PREPARING AN ESTROGEN-DERIVATIVE COMPOUND LABELED WITH Tc-99m AND DETERMINATION OF RADIOPHARMACEUTICAL POTENTIAL

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ABSTRACT

In recent years, estrogen derivative compounds have been used with several purpose which may lead to rapid advances in both diagnosis and therapy of some human cancers.

In this study, an estrogen derivative compound (ESTCPTA) that 3,17-β-estradiolyl propinol attached with 1-(4-methylbenzoic acid)-4,8,11- tetraazacyclotetradecane (CPTA) was synthesized at five steps. Synthesized product was purified by recrystallization in ethyl alcohol. Characterizations of purified products were made by NMR and IR spectroscopy. Synthesized compound was labeled with Tc-99m. RTLC (Radio Thin Layer Chromatography) and radio-paper electrophoresis were used to determine radiochemical yields. Specific activity was approximately 23.7 GBq/mmol and labeling yield was over 95%.

Biodistribution studies were performed on female Albino Wistar rats. Rats were sacricified by ether narcotization at certain time intervals and the organs were removed. Their activities were counted by a gamma counter. The activity per gram tissue was calculated and time activity curves were generated.

INTRODUCTION

The dependence of human breast cancer on hormones has been recognized for nearly a century (1, 2, 5). For recent years, estrogen group compounds have been used different purpose which may lead to rapid advances in both diagnosis and therapy of human cancer (1, 4, 6, 9, 10). Several groups have been studied for synthesis of estrogen derivatives and labeling with some radionuclides such as I-123, I-125, Tc-99m, Re-186, Re-188 (1, 2, 4 - 6, 9). Desombre et al., have been studied some estrogens labeled with Auger emitters purpose of therapy of estrogen receptor positive cancers (1-4). Rijks et al, investigated estrogen receptors in primary and metastatic breast cancer patients with iodine-123-labeled Z-MIVE (cis-11 β-methoxy- 17 α-iodovinylestradiol (7). Jonson and Welch prepared F-18 labeled estrogens and progestins (3). Sasaki et al investigated biodistribution and breast tumor uptake of 16α-[^{18}F]-fluoro-17β-estradiol in rat (8). The aim of this work is to prepare 1-(4-methylbenzoic acid)-4,8,11-tetraazacyclotetradecane (CPTA) attached to ethynyl estradiol as a Tc-99 labeled estrogen derivative compound.

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MATERIALS AND METHODS

Materials: $Na^{99m}TcO_4$ was obtained from Department of Nuclear Medicine of Ege University. Ethynil Estradiol was a gift from Schering Alman Ilac ve Ecza Ticaret Limited. Diphenilethylamine (DIPEA), bromodimethyl ether, 1,4,8,11 tetraazacyclotetradecane, dimethyl formamide were purchased from Aldrich Chemical Co. All other chemicals were supplied from Merck Chemical Co.

Synthesis Procedures: Ethynylestradiol (0.1082 g) was dissolved 20 mL tetrahydrofurane in a 250 mL two-necked round-bottomed flask which have magnetic stirrer. Diphenilethylamine (DIPEA) and 0.0601 mL bromodimethylether were added the solution, then it was refluxed on a water bath for three hours and cooled to room temperature. The purpose of this step is to react ethynyl estradiol with bromomethylmethylether to protect the hydroxyl group.

The reaction mixture was cooled at – 78 °C and 0.0396 mL n-buthyl lithium was added to the mixture than it stirred by magnetic stirrer for 30 minutes. Later 0.0118 g paraformaldhyde was added to the solution then the mixture stirred until cooling to room temperature.

Synthesis of CPTA: 1,4,8,11-tetraazacylotetradecane (0.0701 g) was dissolved in 15 mL ethanol and 3 mL of water, and then lithium hydroxide (0.0034 g) and 4-(bromomethyl)benzoic acid (0.0746 g) dissolved in 4 mL of water were added which the reaction mixture was cooled on ice. The solution was then refluxed for 3.5 h, cooled to the room temperature and ethanol was evaporated. The excess of 1,4,8,11-tetraazacylotetradecane in the alkaline aqueous solution was then extracted with chloroform. 2 µL of Thionyl chloride was placed in a two-necked round-bottomed flask, equipped with a dropping funnel containing CPTA solution which was given drop by drop then the mixture refluxed gently. The mixture refluxed for a further 30 minutes to expel the dissolved sulfur dioxide and allowed to cool. 3-(17-β-estradiol)-propinol was added to the solution in two-necked round-bottomed flask, equipped with a mechanical stirrer and reflux condenser. Completed the reaction by heating on a water bath for 3 hours, when hydrogen chloride is slowly evaluated. Crude product was purified by recrystallization in ethanol. Melting Points are: Ethynyl estradiol: 182-183 °C, CPTA: 184-186 °C, ESTCPTA: 111-115 °C.

ITLC (Instant Thin Layer Chromatography) was performed with a Sigma ITLC chamber supply using cellulose coated plastic sheets (Merck 5565). Merck 20x20 cm cellulose coated plastic sheets with a thickness of 0.1 mm were cut into a smaller 10x1.5 cm sheets and these were used as ITLC support material.

ITLC chromatograms were obtained for cold products and these were exposed under iodine vapor.

^{1}H-NMR, ^{13}C-NMR (in DMSO) and IR spectra were taken to identify the chemical structure.

Labeling Procedure: Ligand was dissolved in 1 mL ethanol. 10 µL Tween-80 and 200 µL serum physiological were added to 10 µL of this solution respectively then 200 µL $SnCl_2$ (1 mg $SnCl_2$ in 1mL 0.1 N HCl) solution was added after 5 mCi Tc-99m. pH was adjusted to 6.5 by

0.1 N ammonia solution then 3 mL serum physiological was added. Reaction mixture was allowed for 20 minutes. Then it was purified by Sep-Pak ® Plus C18 (Waters) and eluted with 6 N NaOH.

Quality controls by performed by RTLC and paper electrophoresis. Labeling yield was over 95%. Average specific activity was approximately 23.7 GBq/mmol (37 MBq/mg).

RTLC Procedures: Every RTLC sheet was covered by cello-band after its development and was cut into 0.5 cm wideness. Then those were counted by using a Cd(Te) detector equipped with a RAD 501 single-channel analyser. RTLC choromatograms were obtained from these figures by plotting counts versus distance. R_f values and labeling efficiencies were gotten from these figures.

Electrophoresis Procedures: Electrophoresis was done with a Gelman Electrophoresis Chamber supply using cellulose acetate strips. Cathode and anode poles and application points were indicated on cellulose acetate strips and these strips were moistened by buffer solution (10 mL Pyridin/0.8 mL Acetic Acid/250 mL H_2O). They were placed in electrophoresis chamber after the samples set on the strips. Standing time and applied voltage for two hours and 250 volts. Developed strips were dried and cut into one cm pieces. They were counted by a Cd(Te) detector equipped with a RAD 501 single-channel analyser.

Measurement of the n-Octanol/Water Partition Coefficient: A 50 µL aliquot of labeled sample was mixed with a 3 mL each of 1-octanol and 0.1 M phosphate buffer (pH 7.4) in a test tube. The tube was vortexed (3x1 min), incubated for 1 hour at room temperature, and then centrifuged for 5 minutes. The 0.5 mL aliquots of each phase were removed and counted by a Cd(Te) detector equipped with a RAD 501 single-channel analyser.

Biodistribution Studies on Rats: The protocol was approved by the Institutional Animal Review Committee. Tc-99m labeled product has been sterilized by passing through a 0.22 µm membrane filter. Then it has been injected from tail vein of female Albino Wistar Rats which were 24 weeks age and 150-200 g weight. Four rats were used for each point of the experiments. The rats were sacrificed under ether narcotization in an ether atmosphere after certain times and their organs were removed. Their activities were counted by a Cd(Te) detector equipped with a RAD 501 single-channel analyser.

Differences in the mean values were evaluated by one-way factorial analysis of variance (ANOVA) for statistical analysis. A linear regression analysis was made for the correlation study. Probability values <0.05 were considered to be significant.

RESULTS AND DISCUSSION

n-Octanol/Water partition coefficient of complexes was 8.07±0.42 (n=3). As expected, the attachment of a lipophilic side chain increased according to ethynyl estradiol ($P_{o/w}$ =3.74 for ethynyl estradiol) (11).

Biodisribution as % dose/g tissue for all studied organs are given in table-1 and some selected ER-rich tissues are given figure-1. ESTCPTA uptake by the uterus, an ER-rich tissue, was

highly selective and it reached 1.244 ± 1.673 % dose/g showing a maximum at 30 minutes. These results agree with other studies carried out other estrogen derivative compounds (3). Ovary and breast showed similar biodistribution profile and relationship between each organ were found statistically significant ($r=0.99$, $p<0.001$). Muscle, breast and uterus tissues showed similar biodistribution profiles. Biodistribution profiles was the same after 60 minutes between ovary and muscle, and breast and uterus. On the other hand there was a significant relation between pancreas and breast ($r=0.98$, $p<0.002$) and pancreas and ovary ($r=0.98$, $p<0.001$). There was also significant relation between fat and L. intestines ($r=0.95$, $p<0.013$), fat and lungs ($r=0.94$, $p<0.016$), fat and spleen ($r=0.95$, $p<0.011$), fat and stomach ($r=0.90$, $p<0.035$), kidney and liver ($r=0.93$, $p<0.022$), L. intestines and lungs $r=0.95$, $p<0.013$, lungs and liver ($r=0.95$, $p<0.013$), stomach and lungs ($r=0.88$, $p=0.047$). Uptake in breast increased while decreasing pancreas uptake at 30th minutes and breast uptake decreased while pancreas uptake increased. An increasing uptake up to 2.876 % dose/g was seen in fat tissue within 180 minutes. Uptake in ER negative tissues such as spleen, heart, liver, brain, stomach, lungs were much less than ER-positive tissues. Liver and kidneys, primary organs of metabolism and excretion of estrogen, take up a great deal of ESTCPTA particularly soon after the injection, it increased slightly within 180 minutes then slightly decreased. ESTCPTA uptake in rat breast was higher that that in ER-negative tissues.

Brain has not showed a significant uptake. That was the expected result although ^{99m}Tc-ESTCPTA has a neutral and lipophilic structure since its molecular weight is over than 500 Dalton. However advised molecular weight of a brain agent should be lower than 500 Dalton.

As a result, we observed high ^{99m}Tc-ESTCPTA uptake by the uterus which is an ER-rich tissue and observed low ^{99m}Tc-ESTCPTA uptake by ER-negative tissues. Therefore we suggest ESTCPTA uptake by the uterus is ER mediated while that by ER-negative tissues is not.

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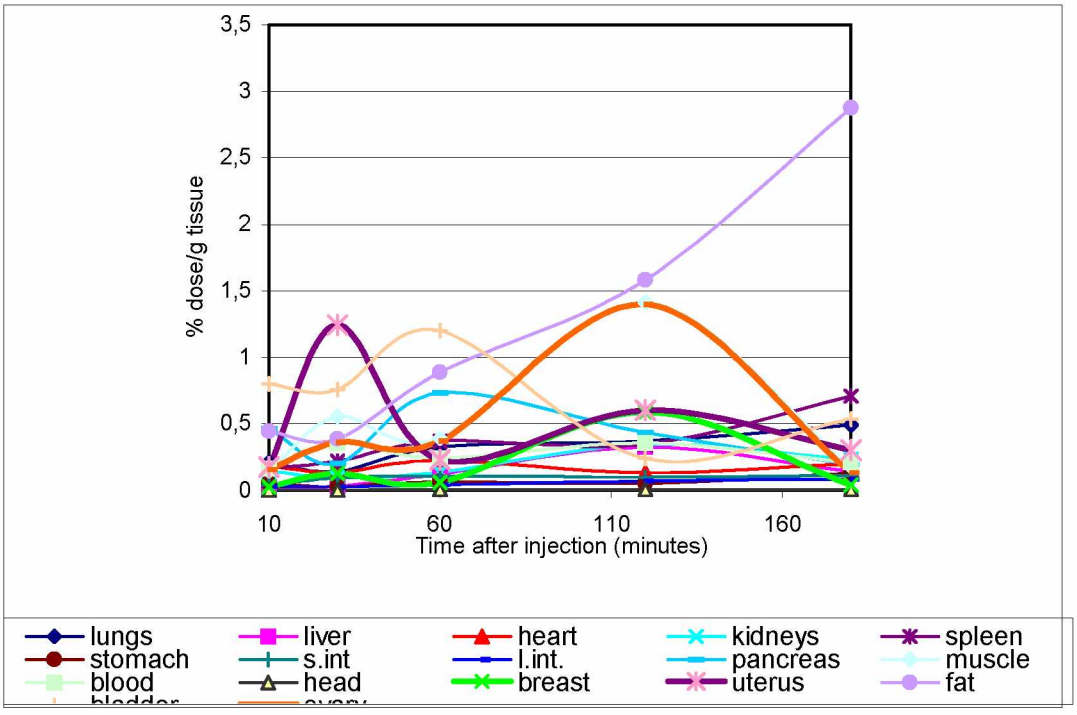

Figure-1 Biodistribution of ESTCPTA on some selected organs.