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Synthesis and Biodistribution Studies of Two Novel Radiolabeled Estrone Derivatives

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Abstract. *Background: Two ^{99m}Tc-DTPA attached estrone derivatives were synthesized and their radiopharmaceutical potential was determined using female albino Wistar rats. Materials and Methods: Two novel radiolabeled estrone derivatives, ^{99m}Tc-2,2',2'',2'''-(2,2'-(2-(3-methoxy-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-2-ylamino)-2-oxoethylazanediyl) bis(ethane-2,1-diyl))bis(azanetriyl) tetraacetic acid (^{99m}Tc-2-DTPA-3-methoxy estrone) and ^{99m}Tc-2,2',2'',2'''-(2,2'-(2-(3-methoxy-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-4-ylamino)-2-oxoethylazanediyl) bis(ethane-2,1-diyl))bis(azanetriyl)tetraacetic acid (^{99m}Tc-4-DTPA-3-methoxy estrone) were synthesized starting from estrone (3-hydroxy-13-methyl-7,8, 9,11,12,13,15,16-octahydro-6H-cyclopenta[a]phenanthren-17(14H)-one) and DTPA anhydride (2-(bis(2-(2,6-dioxomorpholino)ethyl)amino)acetic acid) as potential estrogen receptor imaging agents. The products were crystallized in ethyl alcohol (95%), purified by high performance liquid chromatography (HPLC) and characterized by nuclear magnetic resonance (NMR) and infrared spectroscopy (IR). The effect of the radiolabeled compounds on the biological behaviour of the molecules was evaluated through biodistribution studies in female albino Wistar rats. The rats were sacrificed at various time intervals, their organs were removed, and the activities of organs were counted using a gamma counter equipped with a Cd(Te) solid state detector. Results and Conclusion: Organ uptake was calculated as activity/gram tissue and time versus activity curves were generated. The tissue distribution studies exhibited a receptor-mediated uptake in the target organs of the rats for each compound. Both ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone were stable in vitro and were mainly excreted through the hepatobiliary pathway. The biological data showed that the ^{99m}Tc-2-DTPA-3-methoxy estrone had higher uptake in the target tissues than the ^{99m}Tc-4-DTPA-3-methoxy estrone. The favourable in vitro/in vivo stability and biodistribution profiles suggest that these radioligands are good candidates for further exploration of their potential clinical applications.*

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Key Words: Estrone derivatives, ^{99m}Tc, estrogen receptor, DTPA.

Estrogen compounds are important tools in medicine, especially for estrogen receptor (ER) positive breast and uterine carcinomas and with respect to the preparation of radiopharmaceutics for imaging and treatment (1-6, 7). The determination of estrogen receptor status in human tumors has been shown to improve the selection of patients who may benefit from hormonal therapy and to predict prognosis in patients with primary breast carcinomas.

Several radiolabeled estrogen derivatives have demonstrated highly specific uptake by ER(+) human breast tumors (5-10).

In diagnostic nuclear medicine, ^{99m}Tc has been preferred for most applications, since it is a short-lived radionuclide, present in chemically microscopic amounts and has minimally radioactive emission that is close to optimal for use with today's imaging instruments (1). ^{99m}Tc labeled radiopharmaceuticals have been used for different purposes and ^{99m}Tc-(2-(bis(2-(2,6-dioxomorpholino)ethyl)amino) acetic acid) (^{99m}Tc-DTPA) has been used in routine clinical applications in nuclear medicine (1-3).

The DTPA derivative of a tamoxifen analogue has been proposed as a hydrophilic agent to decrease the liver and lung uptake and increase the tumor to tissue ratio of the drug to allow better imaging of ER(+) tumors. Biber *et al.* reported that deoxydemethyl homoestradiolyl-diethylene-triamine-pentaacetic acid (^{99m}Tc-ESTDTPA) may potentially be used in breast, ovary, uterus, spleen and pancreas imaging (3). Yoda *et al.* prepared a 2-aminooxyethyliminodiacetic acid conjugated estrone derivative and labeled it with ^{99m}Tc producing in good yield; however the labeled complex did not show high affinity in a mammary tumor (4). In the present

study two ^{99m}Tc-DTPA attached estrone derivatives were synthesized and their radiopharmaceutical potential was determined using female albino Wistar rats.

Materials and Methods

All the reagents were commercially available and were analytical grade. The $^{99m}TcO_4^-$ was obtained from the Nuclear Medicine Department of Ege University (Monrol, Turkey) as a generator eluent. The 3-methoxy-13-methyl-7,8,9,11,12,13,15,16-octahydro-6H-cyclopenta[a]phenanthren -7(14H)-one (estrone, purity >98%) and DTPA anhydride (2-(bis(2-(2,6-dioxomorpholino)ethyl) amino)acetic acid) were purchased from Sigma (D6148-5G).

IR spectra were recorded on a Shimadzu spectrometer, Osman Gazi University, Eskisehir, Turkey. The proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were taken at Ataturk University, Erzurum, Turkey.

ITLC (instant thin layer chromatography) and TLRC (thin layer radio chromatography) were performed with a Sigma ITLC chamber using silica gel coated plastic sheet (Merck 5554 20x20 cm) which were cut into smaller 10x2.00 cm sheets and used as instant or radio TLC support material. The organic phase of benzene/acetone/water (2/1/2; v/v/v) and 0.90% serum physiological was used as the mobile phase for ITLC and TLRC. Each TLRC sheet was covered by a cello band after its development and was cut into 0.50 cm widths. The activity was counted using a Cd(Te) detector. The chromatograms were obtained by plotting count versus distance and the Rf values and labeling efficiency were derived from these figures. The Rf values of the cold products were obtained by spraying of concentrated sulfuric acid over the ITLC sheets and leaving them for 10 min at 75˚C.

Electrophoresis was conducted with a Gelman electrophoresis chamber. The cathode and anode poles and application points were indicated on the cellulose acetate strips which were moistened by buffer solution (pyridine/acetic acid/water; 10/0.80/250; v/v/v). After each compound was set on the strips, they were placed in the electrophoresis chamber. The standing time and applied voltage were 2 hours and 250 volts, respectively. The developed strips were dried and cut into 1-cm pieces and each one was counted in the Cd(Te) detector. Movement was determined relative to pertechnetate and hydrolyzed Tc-99m colloid.

Synthesis procedures

2,2',2'',2'''-(2,2'-(2-chloro-2-oxoethyla-zanediyl)bis(ethane-2,1-diyl))bis(azanetriyl)tetraacetic acid. In the first stage the hydroxyl group of DTPA anhydride was converted to the chloride derivative of DTPA (Figure 1). Forty μL (0.55 mmol) thionyl chloride in hexane (1 mL) was added to 0.50 mmol DTPA anhydride in hexane (10 mL) drop by drop within 20 minutes, the mixture was heated at reflux for 30 minutes, the solvent was removed and 2,2',2'',2'''-(2,2'-(2-chloro-2-oxoethylazanediyl)bis(ethane-2,1-diyl))bis(azanetriyl)tetraacetic acid (substance A) was obtained.

2,2',2'',2'''-(2,2'-(2-(3-methoxy-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-4-ylamino)-2-oxoethylazanediyl) bis(ethane-2,1-diyl))bis(azanetriyl)tetraacetic acid (4-DTPA-3-methoxy estrone). Firstly 4-amino-3-methoxy-13-methyl-7,8,9,11,12,13,15,16-octahydro-6H-cyclopenta[a]phenanthren-17(14H)-one (substance B) was prepared from 3-hydroxy-13-methyl-7,8,9,11,12,13,15,16-octahydro-6H-cyclopenta[a]phenanthren-17(14H)-one (estrone) as previously described (11). 0.45 mmol NaOH in 2 ml distilled water was added to the 0.45 mmol of substance A in 10 mL THF (Tetra Hydro Furan) and the solution was cooled to about 0-5˚C in a salty ice-bath. Then 0.43 mmol of substance B in 10 mL THF at 0-5˚C was added (Figure 2). The mixture was stirred for 30 minutes at the same temperature, allowed to reach room temperature and then the solvent was removed under reduced pressure. Two mL of 0.01 N HCl were added to the residue dissolved in ethyl alcohol (95%), and the precipitate was filtered. The 4-DTPA-3-methoxy estrone (substance D) was recrystallized with ethyl alcohol (95%) and the yield was 0.197 g (65%).

The characteristics of 4-DTPA-3-methoxy estrone were: IR(KBr, cm^{-1}) 3407(amide, NH), 2956(alkyl, CH), 1743 (ketone, C=O), 1633 (alkenyl, C=C) and 1H-NMR (DMSO) δ_H 0.90(s, 3H, 13-CH3), 1,72(m, 1H, 9-H), 1.73(m, 2H, 15-H), 1.80(t, 2H, 12-H), 1.82(m, 2H, 8-H), 1.89(m, 1H, 14-H), 2.35(m, 2H, 11-H), 2.52(t, 2H, 16-H), 2.58(t, 2H, 7-H), 2,63(t, 2H, 20-H), 2.72(t, 2H, 19-H), 2.87(m, 1H, 10-H), 2.98(s, 2H, 21-H), 3.40(s , 2H, 18-H), 3.61(s, 3H, 3-OCH_3), 6.85(m, 1H, 2-H), 6.92(d, 1H, 1-H), 8.90(s, 1H, NH) and 10.10(s, 1H, COOH) 13C-NMR (DMSO): δ_H 17.7, 19.9, 24.3, 24.6, 26.7, 31.3, 32.3, 33.8, 35.1, 40.4, 43.9, 55.4, 55.8, 56.57, 57.2, 57.6, 59.4, 73.4, 75.6, 109.8, 122.4, 127.4, 132.2, 133.2, 144.13, 166.1, 166.1, 166.1, 166.1, 168.46, 215.6.

2,2',2'',2'''-(2,2'-(2-(3-methoxy-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a] phenanthren-2-ylamino)-2-oxoethylazanediyl) bis(ethane-2,1-diyl))bis(azanetriyl) tetraacetic acid (2-DTPA-3-methoxy estrone). 2-DTPA-3-methoxy estrone (substance E) was prepared with similar procedure that was described for 4-DTPA-3-methoxy estrone (substance D). 2-amino-3-methoxy-13-methyl-7,8,9,11,12,13,15,16-octahydro-6H-cyclopenta[a] phenanthren-17(14H)-one (substance C) was synthesized from 3-hyroxy-13-methyl-7,8,9,11,12,13,15,16-octahydro-6H-cyclopenta[a]phenanthren-17(14H)-one as described previously (11). Then 0.45 mmol substance A and 0.43 mmol substance C were used to produce 0.195 g (67%) 2-DTPA-3-methoxy estrone, (substance E) as shown in Figure 3.

The characteristics of 2-DTPA-3-methoxy estrone were: IR(KBr, cm^{-1}) 3419(amide, NH), 2967(alkyl, CH), 1743 (ketone, C=O), 1633 (alkenyl, C=C) and 1H-NMR (DMSO) δ_H 0.90(s, 3H, 13-CH_3), 1,65(m, 2H, 8-H), 1.73(m, 2H, 9-H), 1.76(m, 2H, 15-H), 1.80(t, 2H, 12-H), 1.90(m, 1H, 14-H), 2.31(m, 2H, 11-H), 2.46(t, 2H, 16-H), 2.70(t, 2H, 20-H), 2.78(t, 2H, 19-H), 2.80(t, 2H, 7-H), 2,92(m, 1H, 10-H), 3.10(s, 2H, 21-H), 3.40(s, 2H, 18-H), 3.70(s, 3H, 3-OCH_3), 6.60(s, 1H, 4-H), 7.10(s, 1H, 1-H), 9.15(s, 1H, NH) and 10.12(s, 1H, COOH), ^{13}C-NMR (DMSO): δ_H 18.60, 26.15, 26.90, 28.40, 30.01, 32.23, 32.50, 32.60, 36.15, 41.40, 46.80, 48.85, 52.56, 56.62, 58.54, 63.20, 112.82, 117.85, 130.24, 132.21, 138.54, 155.50, 168.93, 180.31, 213.59.

Labeling procedure. To prepare the ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone, 0.03 μmol of 2-DTPA-3 or 4- DTPA-3-methoxy estrone was dissolved in 1.00 mL ethyl alcohol (80%). Ten μL Tween-80 and 200 μL physiological serum were added to the prepared solutions and stirred for about 5 min. Then 185 MBq (5.0 mCi) $^{99m}TcO_4^-$ and 200 μL $SnCl_2$ (1mg $SnCl_2$ in 1mL 0.1 N HCl) solutions were added to the ligand solution and the reaction was allowed to proceed at 80-90˚C for 30 min. Finally, the pH was adjusted to 6.5 by 0.01 N sodium hydroxide

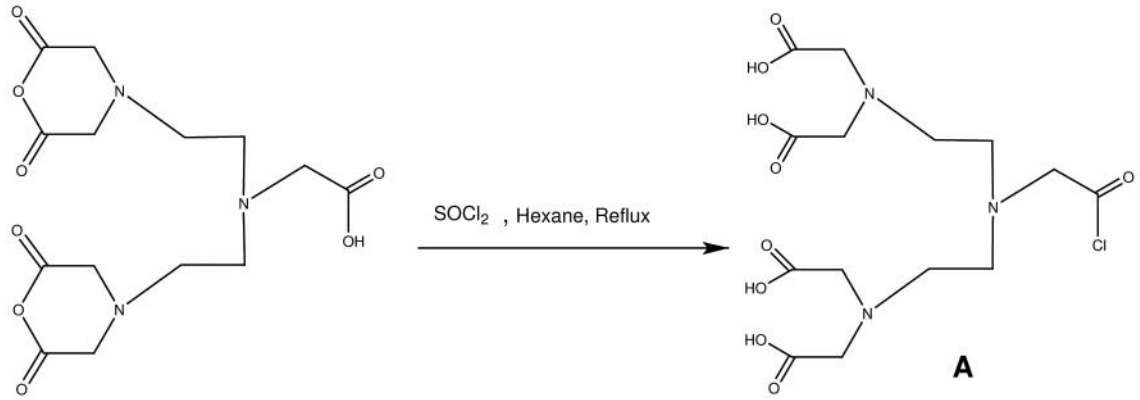

Figure 1. *Synthesis of 2,2',2'',2'''-(2,2'-(2-chloro-2-oxoethylazanediyl)bis(ethane-2,1-diyl))bis(azanetriyl)tetraacetic acid.*

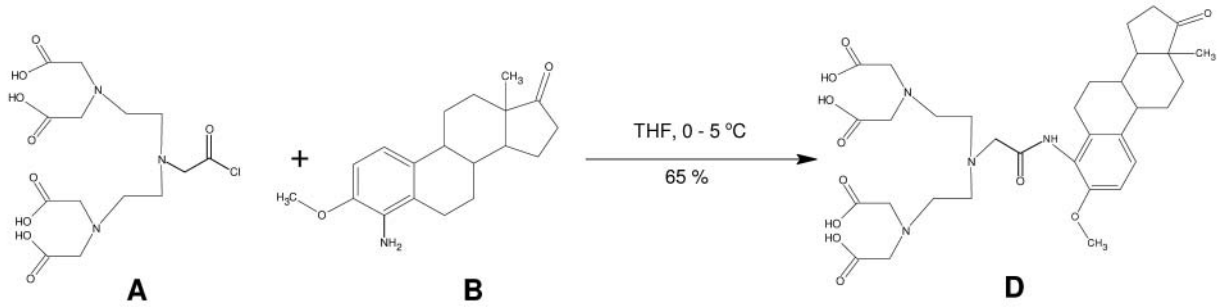

Figure 2. *Synthesis of 4-DTPA-3-methoxy estrone.*

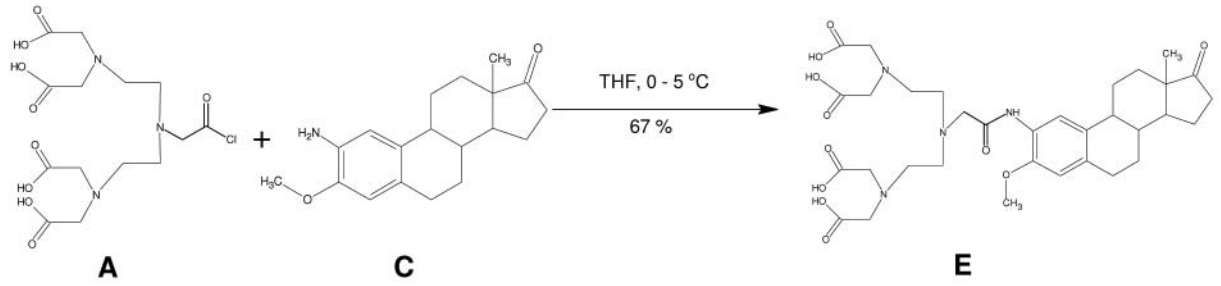

Figure 3. *Synthesis of 2-DTPA-3-methoxy estrone.*

solution. Quality controls were performed by TLRC. The labeling yield was over 95%. Estimated specific activities were approximately 6.10 GBq/μmol (166 mCi/μmol).

Stability in serum. The *in vitro* stability of ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone in serum was determined by incubating 0.60 mL of each complex solution with 1 mL of human serum at 37˚C. Aliquots were then analyzed 1, 2, 3, 4 and 24 h by TLRC and the radioactivity was counted using a Cd(Te) detector.

Biodistribution studies in albino Wistar rats. The experiments with animals were approved by the Institutional Animal Review Committee of Ege University. The biodistribution data were expressed as a percentage of the injected radioactivity per gram of tissue for the selected organs as the mean value of three rats.

Three albino female Wistar rats weighing approximately 90-135 g were used for each time point of the experiment. After sterilization of the ^{99m}Tc labeled products by means of passing through a 22 μm membrane filter, the active substances were injected into the tail vein of the rats. A separate group of animals was co-injected with 10.0 μg unlabeled estrone derivatives (per animal) 2 h before the radiotracer for the receptor saturation studies. After injection with radiotracer the animals were sacrificed under an intense ether atmosphere at 10, 60 and 120 min and the tissues of interest were removed. All the tissues were weighed and counted for radioactivity with a Cd(Te)

Table I. *Tissue distributions of ^{99m}Tc-2-DTPA-3-methoxy estrone as percentage of injected dose/g organs in rats.*

Organs	10 minutes		60 minutes		120 minutes	
	ER unsat.	ER sat.	ER unsat.	ER sat.	ER unsat.	ER sat.
Lungs	0.56±0.43	0.36±0.30	0.11±0.02	0.11±0.03	0.09±0.03	0.11±0.03
Liver	0.09±0.04	0.13±0.07	0.06±0.01	0.08±0.03	0.09±0.01	0.06±0.01
Heart	0.94±0.99	0.12±0.06	0.07±0.01	0.10±0.02	0.09±0.03	0.09±0.02
Stomach	1.79±0.52	0.15±0.03	3.29±0.19	2.80±1.57	4.21±0.58	5.24±0.44
Kidneys	0.30±0.21	0.25±0.17	0.13±0.01	0.14±0.04	0.23±0.16	0.10±0.06
Spleen	0.08±0.03	0.18±0.08	0.10±0.04	0.08±0.02	0.08±0.02	0.10±0.01
Pancreas	0.31±0.13	0.41±0.29	0.14±0.04	0.11±0.02	0.08±0.02	0.15±0.03
S.Intestine	0.17±0.02	0.08±0.01	0.06±0.02	0.82±0.74	0.16±0.03	0.51±0.32
L.Intestine	0.10±0.04	0.06±0.05	0.06±0.01	0.13±0.12	0.26±0.04	0.42±0.28
Blood	0.80±0.60	0.36±0.03	0.16±0.03	0.34±0.11	0.24±0.04	0.49±0.17
Head	0.02±0.01	0.03±0.02	0.02±0.00	0.01±0.00	0.02±0.00	0.01±0.00
Breast	0.14±0.01	0.09±0.06	0.10±0.01	0.07±0.02	0.13±0.05	0.10±0.03
Muscle	0.15±0.07	0.24±0.04	0.09±0.04	0.63±0.41	0.10±0.07	0.14±0.08
Uterus	0.18±0.11	0.09±0.05	0.10±0.01	0.10±0.04	0.13±0.05	0.12±0.04
Fat	0.46±0.30	0.26±0.08	0.48±0.35	0.22±0.06	0.15±0.04	0.33±0.06
Ovary	0.28±0.23	0.12±0.08	0.27±0.24	0.12±0.08	0.06±0.01	0.02±0.02
U. Bladder	0.51±0.17	1.23±0.95	1.67±1.71	0.62±0.33	0.29±0.08	1.26±1.12

Table II. *Tissue distributions of ^{99m}Tc-4-DTPA-3-methoxy estrone in rats in percentage of injected dose/g organs.*

Organs	10 minutes		60 minutes		120 minutes	
	ER unsat.	ER sat.	ER unsat.	ER sat.	ER unsat.	ER sat.
Lungs	0.38±0.16	0.01±0.00	4.94±0.01	0.01±0.01	1.28±1.53	0.01±0.00
Liver	1.03±0.57	0.00±0.00	1.72±0.00	0.00±0.00	4.30±4.97	0.02±0.02
Heart	0.04±0.01	0.01±0.01	0.07±0.01	0.01±0.01	0.04±0.04	0.01±0.01
Stomach	0.05±0.03	0.01±0.00	0.02±0.01	0.01±0.01	0.02±0.02	0.00±0.00
Kidneys	0.07±0.00	0.02±0.01	0.12±0.00	0.02±0.00	0.07±0.05	0.03±0.00
Spleen	0.75±0 .28	0.03±0.01	1.06±0.00	0.01±0.01	0.03±0.01	0.02±0.01
Pancreas	0.04±0.03	0.03± 0.01	0.08±0.01	0.02±0.01	0.05±0.01	0.04±0.02
S.Intestine	0.01±0.01	0.01±0.01	0.09± 0.02	0.03±0.02	0.03±0.01	0.01±0.00
L.Intestine	0.02±0.01	0.01±0.0	0.09±0.00	0.01±0.00	0.03±0.01	0.04±0.02
Blood	0.08±0.01	0.03±0.03	0.06±0.02	0.04±0.02	0.08±0.04	0.04±0.02
Head	0.01±-	0.01±0.00	0.01±0.00	0.00±0.00	0.01±0.00	0.00±0.00
Breast	0.01±-	0.04±0.04	0.01±0.02	0.02±0.02	0.05±0.04	0.03±0.02
Muscle	0.04±0.02	0.09±0.04	0.05±0.02	0.04±0.02	0.12±0.09	0.11±0.01
Uterus	0.04±0.01	0.02±0.01	0.06±0.01	0.01±0.01	0.05±0.01	0.03±0.01
Fat	0.06±0.07	0.09±0.07	0.06±0.03	0.07±0.03	0.17±0.10	0.12±0.06
Ovary	0.02±0.01	0.01±0.01	0.11±0.01	0.01±0.01	0.07±0.04	0.04±0.03
U. Bladder	0.13±0.02	0.34±0.21	0.22±0.10	0.17±0.10	0.76±0.61	0.77±0.65

detector. The percent of radioactivity per gram of tissue weight (in % Injected Dose (ID)/g organs) was determined.

Statistical analyses. Differences in the mean values of the measured activities were evaluated statistically by univariate analysis of variance. Two-tailed Pearson's correlation was applied. The level of significance was set below $p<0.05$.

Results

The quality control of the radiolabeled compounds was performed by TLRC and electrophoresis. According to TLRC results pertechnetate moved with the solvent front and the reduced hydrolyzed ^{99m}Tc remained at the origin in

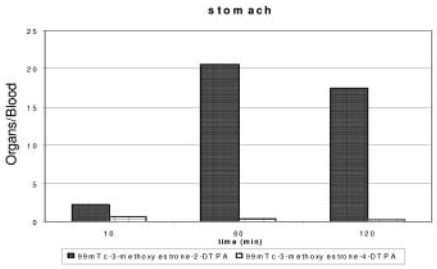

Figure 4. *Stomach uptake of ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone.*

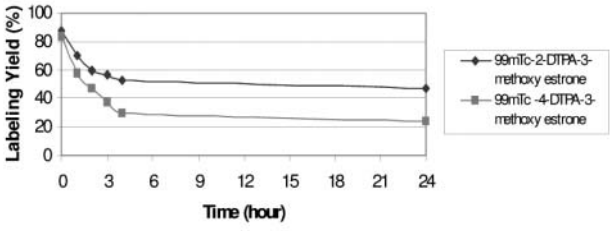

Figure 5. *In vitro stability in serum.*

the solvent; Rf values were between 0.29 and 0.49 for each compound. In electrophoresis, both pertechnetate and ^{99m}Tc-DTPA moved to towards to the anode while the ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone stayed at application point. Thus the labeled compounds have neutral structures.

The time-dependent biodistribution data in the organs of the rats are presented in Tables I and II for ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone, respectively. The radiolabeled substances showed a marked uptake in the ER-containing uterus, ovary and breast of the rats and the concentration in the ovary was higher than the others. The uptake of ^{99m}Tc-2-DTPA-3-methoxy estrone in the target tissues, especially at 10, 60 and 120 min post injection was higher than the corresponding ^{99m}Tc-4-DTPA-3-methoxy estrone uptake.

The organs involved in steroid hormone metabolism and excretion (the liver, intestine and kidney) showed high uptake of radioactivity. The uptake of radioactivity in the stomach over time was found to be the highest for ^{99m}Tc-2-DTPA-3-methoxy estrone 5.24 % ID/g 120 min post injection. Different uptake profiles for some organs were found between the two isomers. For instance, ^{99m}Tc-2-DTPA-3-methoxy estrone uptake in the pancreas was much higher than ^{99m}Tc-4-DTPA-3-methoxy estrone uptake although in the spleen uptake of ^{99m}Tc-4-DTPA-3-methoxy estrone was higher than ^{99m}Tc-2-DTPA-3-methoxy estrone uptake over the period of study.

The stomach uptake ratio of ^{99m}Tc-2-DTPA-3-methoxy estrone to ^{99m}Tc-4-DTPA-3-methoxy estrone isomers ratio was high and reached 2658 within 120 min (Figure 4). Statistical analyses demonstrated both of the labeled ligands were receptor specific for the lung, spleen and uterus. Both of the radiolabeled ligands were metabolized in the same way in the ovary, lung, liver, heart, however there were distinct differences between the ligands in most of the other organs such as the stomach, kidneys, pancreas, intestines, breast and uterus according to the statistical analyses ($p<0.05$).

Figure 5 presents the stability in human serum of each compound. Figure 6 and Figure 7 demonstrate the organ/blood ratios of ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone. The 4-DTPA derivative showed the highest organ/blood ratios in the lung, liver and spleen while the 2-DTPA derivative had the highest ratio in the stomach. Figure 8 represents the organ/muscle ratios of ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone which were similar to the organ/blood ratios. In addition, similar biodistribution ratios were found in the receptor saturated studies.

In the case of ER-saturation following co-injection of cold 2-DTPA-3-methoxy estrone, most of the tissues such as the lungs, liver and spleen showed similar uptakes with normal distribution but the same organs did not show similar uptakes with 4-DTPA-3-methoxy estrone. The receptor unsaturated/receptor saturated ratio of 4-DTPA-3-methoxy estrone was raised to 128 and 215 for the liver and lungs within 120 min, respectively, but the same organ values did not change with 2-DTPA-3-methoxy estrone.

Discussion

The ratios of ^{99m}Tc-2-DTPA-3-methoxy estrone uptake to ^{99m}Tc-4-DTPA-3-methoxy estrone uptake in the stomach were 253 and 2658 for receptor unsaturated to unsaturated and for saturated to saturated studies, respectively. Receptor saturation led to up ten times more uptake of the for ^{99m}Tc-2-DTPA-3-methoxy estrone derivative. This may due to other mechanisms other than receptor saturation playing a role in the uptake of ^{99m}Tc-2-DTPA-3-methoxy estrone derivative in the stomach. The ^{99m}Tc-2-DTPA-3-methoxy estrone was more stable in serum than the ^{99m}Tc-4-DTPA-3-methoxy estrone as seen in Figure 5. For that reason higher uptake in stomach was not due to *in vivo* decomposition of 2-isomer. Similar results were found with the ^{131}I labeled 2-iodo-methoxy-estron derivative (11) and ^{131}I-Tamoxifen (12).

Different organ uptakes were seen between the two isomers, this may not only be lipophilicity, but also to the molecular structure and the position of the attached radionuclide which are crucial for the selective binding to ERs. Hanson *et al.* have reported that ortho, para and meta

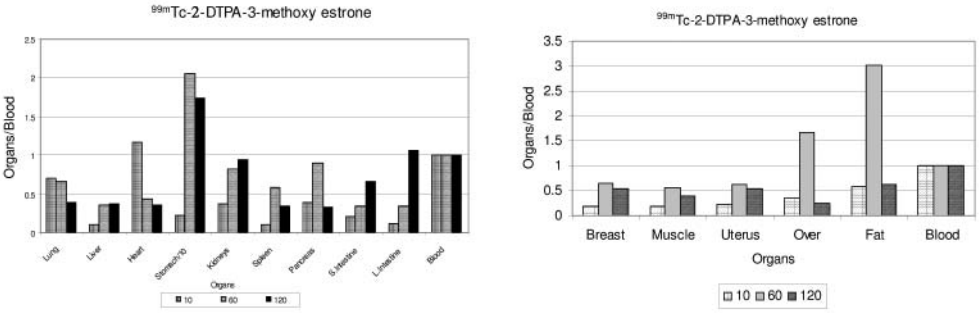

Figure 6. *Organ/blood ratios for ^{99m}Tc-2-DTPA-3-methoxy estrone.*

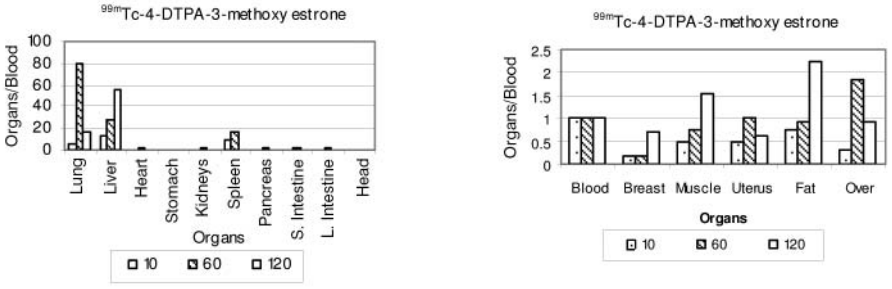

Figure 7. *Organ/blood ratios for ^{99m}Tc-4-DTPA-3-methoxy estrone.*

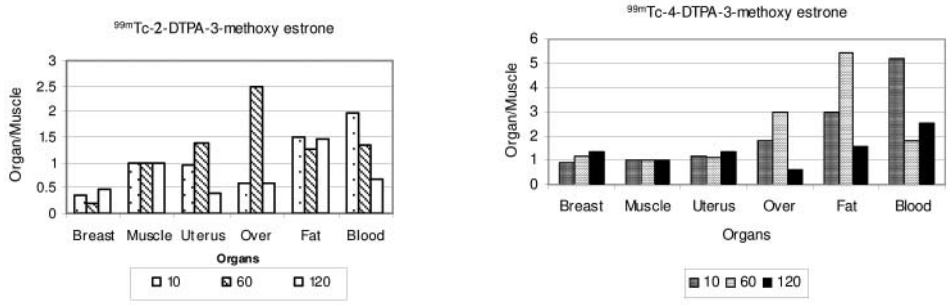

Figure 8. *Organ/Muscle ratios for ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone.*

isomers of trifluoromethylphenylvinyl estradiols possessed different relative binding affinities (RBA) to estrogen receptors (13). The meta and para isomers had lower RBA values, while the ortho isomer had the highest RBA value compared to estradiol (2). Some other reports have indicated that the stereochemistry of the 17α substituent in the 17α-iodovinyl estradiol was important in the receptor binding affinity of the agent (14). For instance, it has been demonstrated that Z-isomers of 17α-iodovinyl estradiols bind to receptors more effectively than the corresponding E-isomers (14, 15). Sasaki *et al.* postulated that, in molecules such as Z-17α-iodovinyl-estradiol, the large iodine atom was directed to a different portion of the receptor than the iodine in the corresponding E-isomer Z-17α-iodovinyl-estradiol. Furthermore, ^{123}I labeled Z-17α-iodovinyl-11β-chloromethyl-estradiol in human breast carcinoma xenographs in mice demonstrated continuous and selective accumulation of the [^{123}I]Z-CMIV in ER positive tumors with a high target/nontarget ratio (5). Silva *et al.* synthesized two novel radiolabeled estrogen derivatives, [^{125}I](E)-3-methoxy-17α-

iodovinylestra-1,3,5(10),6-tetraen-17β-ol (Z[^{125}I]IVDE) and [^{125}I](Z)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (Z[^{125}I]IVDE) (6) and studied the influence of the introduction of a C6-C7 double bond on the biological properties of the estradiol molecule. The Z-isomer, owing to its higher *in vivo* uptake by the target tissue, had the preferable configuration for further development of similar compounds for estrogen receptor detection. The ranges of the uterus/blood and uterus/muscle ratios were approximately between 1 and 3. These ratios were in good agreement with uterus/blood ratios obtained in our previous study (11).

Skaddan *et al.* have synthesized ^{99m}Tc labeled 7α-substituted estradiol complexes (1) and in immature female rats showed low non-receptor-mediated uptake in the uterus and ovaries and high uptake in the nontarget organs such as the liver and fat compared to 16α-[^{18}F]fluoroestradiol (FES). The present results agree with their data regarding uptake by the uterus and ovaries. However, the ^{99m}Tc-DTPA-3-methoxy estrone derivative showed lower liver and fat uptake compared to the ^{99m}Tc labeled 7α-substituted estradiol complexes because of their lower lipophilicity.

Dence *et al.* investigated the biodistribution of 17α-[^{11}C]methylestradiol and 11β-ethyl-17α-[^{11}C]methylestradiol, in rats (15). The uterus and ovaries were target tissues and they demonstrated receptor mediated uptake of the complexes. In their study, uptake in the uterus and ovaries decreased 4.02 and 1.28 times by receptor saturation in 40 min, respectively. In the present study, these ratios were 1.03 and 2.24 in 60 min for ^{99m}Tc-2-DTPA-3-methoxy estrone and 4.94 and 8.88 for ^{99m}Tc-4-DTPA-3-methoxy estrone. The ovaries/muscle ratios decreased 1.44 times by receptor saturation in 40 min for 17α-[^{11}C]methylestradiol, while with ^{99m}Tc-2-DTPA-3-methoxy estrone this ratio was 2.98 in 60 min, and it was 2.50 with ^{99m}Tc-4-DTPA-3-methoxy estrone. The ovaries/blood ratios were 1.65 and 1.84 for ^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone, respectively. These ratios were 1.57 and 1.05 with receptor saturation at the same time intervals in Dence's report and our earlier work, respectively (15-17). Decreasing uterus/blood ratios of 5.04, 2.19, 3.48 and decreasing uterus/muscle ratios of 4.50, 7.35, 4.02 by receptor saturation at the same time intervals were shown in Dence's report (17α-[^{11}C]methylestradiol) and our work (^{99m}Tc-2-DTPA-3-methoxy estrone and ^{99m}Tc-4-DTPA-3-methoxy estrone), respectively. According to these results, the decrease in the ratios of ovaries to muscle and blood of the ^{99m}Tc-DTPA-3-methoxy estrone derivatives was higher than the other ligands while the decrease in ratios of uterus to muscle and blood of the ^{99m}Tc-DTPA-3-methoxy estrone derivatives by receptor saturation was less than 17α-[^{11}C]methylestradiol. The ^{99m}Tc-DTPA-3-methoxy estrone derivatives seem to show higher ovaries/muscle ratios than 17α-[^{11}C] methylestradiol (15-17).

Singh *et al.* have reported that 2-methoxyestrone inhibited 2-deoxy-D-[1-^{3}H]-glucose uptake in MCF-7 breast cancer cells (18). They have also pointed out that 2-methoxyestrogens may exert an anti-mitotic effect on cells by stabilizing microtubules in a similar manner to that of paclitaxel. Since cancer cells are unable to store glucose and depend upon its constant uptake in order to meet their energy requirement, the development of drugs inhibiting glucose uptake could have therapeutic potential for the treatment of breast cancer. According to this consideration ^{99m}Tc-labeled-2-DTPA-3-methoxy estrone may be an outstanding scintigraphic agent to investigate breast cancer.

In conclusion, ^{99m}Tc-2-DTPA-3-methoxy estrone has more stability and significantly higher uptake in target tissues than the ^{99m}Tc-4-DTPA-3-methoxy estrone isomer. The results of this study are sufficiently encouraging to warrant further evaluation of the current and related compounds as possible tumor imaging agents in estrogen-rich tissues.

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