LABELLING OF TAMOXIFEN WITH I-131 BY IODOGEN METHOD

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1. INTRODUCTION

The nonsteroid antiestrogen Tamoxifen is the endocrine treatment modality most widely used in breast cancer[1]. Anti estrogen therapy affects principally ER (Estrogen Receptor) rich tissues. Tamoxifen, the prototypical antiestrogen, has been employed over the course of three decades for the treatment of ER(+) tumors[2]. Both steroidal and nonsteroidal estrogens and antiestrogens tracers containing iodine as the radiolabel have been investigated for this purpose[3]. In the view of this information radiolabeled Tamoxifen ligand would be useful in diagnosis or therapy of diseases that produce high levels of ERs, such as ovarian cancer, endometriosis, uterine carcinoma and meningioma.

There are several studies about radiolabeled Tamoxifen and Tamoxifen derivative compounds with different radionuclides. Among these radionuclides ^{131}I is an attractive one because its wide range of decay properties is well-suited for therapy together with imaging[4].

One of the labeling methods with radioactive iodine is to use an oxidizing agent and to attach iodine via an electrophilic substitution reaction. One of the most commonly used oxidizing agent is 1,3,4,6-tetrachloro-3α, 6α -diphenylglycouril with the trade name of iodogen[5]. This agent was used in the literature for radioiodination procedures of different kind of organic compounds[6,7,8]. Several iodinated Tamoxifen derivatives have been reported in the literature. De Vos et al. synthesized ^{123}I-labeled iodomethyl-N,N-diethyltamoxifen (ITX) for human breast imaging[9].Young et al. investigated the biodistribution and dynamic behavior of iodoxifene with ^{125}I and ^{131}I in rats[10]. ^{18}F-Tamoxifen recently was shown to provide useful information in predicting the effect of Tamoxifen therapy in patients with recurrent or metastatic ER (+) breast cancers. Those tumors that showed good uptake of radiolabeled Tamoxifen gave a positive response to Tamoxifen therapy[11]. To our knowledge, there is no report about ^{131}I radiolabeled Tamoxifen using iodogen as oxidizing agent. Thus this study is aimed to label Tamoxifen with ^{131}I by using iodogen method and investigate its radiopharmaceutical potential in rats.

2. MATERIALS AND METHODS

$Na^{131}I$ was supplied by Monrol, Turkey. Tamoxifen was a gift from Astra Zeneca Ltd. Sti., Istanbul, Turkey. Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenyl glycoluril) was purchased from Sigma. All other chemicals were supplied from Merck Chemical Co and Aldrich Chemical Co.

Synthesis of cold iodinated Tamoxifen (Cold-iodinated-TAM)
Cold-TAM was iodinated with inactive iodine and the product was analyzed with High Performance Liquid Chromatography (HPLC). It was dissolved in 0.01% ethanol and stochiometrically equivalent potassium iodide was added. pH was adjusted to 8 with 1 N NH_4OH.

Labeling procedure
Iodogen method was used for the procedure. Prior to radiolabeling, 100 μg of iodogen (1,2,3,4,6-tetrachloro-3α,6α-diphenylglycoluril) in CH_2Cl_2 solution at a concentration of 1 mg/4 ml was evaporated successively to four reaction vials. The solvent was allowed to evaporate forming a thin solid layer on the wall of the reaction vials. Radiolabeling was done using these vials. 1 ml of Tamoxifen at a concentration of 2mg/10 ml (0.01% ethanol) was added to the first vial. Then the labeling was started by addition of 0.4 ml of $Na^{131}I$ 185 MBq (5 mCi) which was gently mixed and incubated for 5 minutes at normal room temperature (20-25 ^0C) and pH observed at 8. The labeled compound was then transferred to the last vial after being incubated in each vial for 5 minutes at room temperature. Thereafter 0.1 ml (0.2 N Na_2SO_3) solution was added to the last vial to reduce non-incorporated iodine.

Chromatography
High Performance Liquid Chromatography (HPLC)

A low pressure gradient HPLC system (LC-10ATvp quaternary pump and SPD-10A/V UV detector and a syringe injector equipped with a 1 mL loop and VP 250/21 Nucleosil 100-7 C18 column-Macherey-Nagel), was used for preparative procedures. The elute was collected with a FRC-10A fraction collector (Shimadzu) (model LC-10Atvp Shimadzu, Japan). The flow rate was set at 9.0 ml/min. Column was eluted with 60% acetonitrile, 40% water. UV detection was achieved at 240 nm.

Radio electrophoresis procedure

Radio electrophoresis was performed with a Gelman Electrophoresis Chamber supply using cellulose acetate strips. Cathode and anode poles and application points were indicated on the cellulose acetate strips, and these strips were moistened by buffer solution [n-butanol – H_2O – acetic acid (4/2/1)] (pH = 3). After the sample was set on the strips, it was placed in electrophoresis chamber. Standing time and applied voltage were 105 minutes and 300 volts, respectively. The developed strips were dried and cut into one cm pieces. They were counted in a Cd(Te) detector equipped with a RAD 501 single-channel analyzer. The same procedure was applied to ^{131}I and I_2. Data of migration of component and labeling yields were found.

Biodistribution studies on rats

Experiments with animals were approved by the Institutional Animal Review Committee of Ege University. The biodistribution in percentage of injected radioactivity per gram of tissue for some selected organs as the mean value of three rats. They performed on Albino female Wistar rats weighing approximately 130-180 g. For blocking of iodine uptake into the thyroid gland, 10 mg of potassium iodide was added to one liter drinking water of rats.

After ^{131}I labeled compound was sterilized by passing through a 0.22 μm membrane filter, it was injected into the tail vein of the animals (440 ng / each rats). The activity was approximately 7.4 MBq (200 μCi)/rat. Then the rats were sacrificed at 30, 60 and 180 minutes under ether anesthesia and tissues of interest were removed. Blood was taken, organs were excised. All tissues were weighed and counted for radioactivity with a Cd(Te) detector. The percent of radioactivity per gram of tissue weight (in % injected activity / g tissue) was determined.

In vivo blocking experiments

4.4 μg/rat of nonlabeled ligand (Tamoxifen) was prepared under the same conditions as ^{131}I-TAM and injected into the tail vein of the animals 15 minutes before ^{131}I-TAM to determine whether uptake in ER expressing target tissue was specific. The same procedures were repeated as indicated above.

3. RESULTS AND DISCUSSION

The quality control of radiolabeled compound was performed by radio electrophoresis. Table 1 shows the radio electrophoresis diagrams data. Radiochemical yield was 88.56 ± 3.10% (n=8) according to electrophoresis diagrams. The labeling yield was found to be considerably higher with respect to the yield obtained from Stricland et al.'s study[3]. Fig. 1 shows HPLC chromatograms of Tamoxifen and iodinated Tamoxifen. These performed that the iodinated compound is sufficient pure. The obtained results in this study clearly showed that this compound can be successfully radioiodinated using iodogen as an oxidation agent.

According to biodistribution resuts ovary, breast and uterus showed similar biodistribution profiles. Increased uptake was detected for these organs in 60 minutes. Uptake in all ER rich tissues was higher in 180 minutes for ER unsaturated experiments as shown in Fig. 2 and Fig. 3. These data indicate that labeled compound maintain in uterus within 180 min.

The compound has shown ER specifity, it may potentially be used in ER based mammary tumours and endometrial cancer cells for therapy. Brain did not show any significant uptake. Thyroid uptake was checked to verify the in vivo stability of the ^{131}I-TAM, and we did not observe any significant uptake in the thyroid.

4. CONCLUSION

Our results showed that Tamoxifen can be radioiodinated using iodogen method. Measuring tumor uptake of radiolabeled Tamoxifen can provide more accurate information about the effect of antiestrogen therapy. In addition, in vivo imaging of radiolabeled Tamoxifen tumor uptake, retention, and eventually efflux not only can help to increase our understanding of the action of tamoxifen, it also can help to increase our knowledge of the

mechanisms involved in resistance to the drug. Consequently ^{131}I-TAM may be proposed as a therapeutic agent to treat to mammary tumours and endometrial cancer cells.

5. REFERENCES

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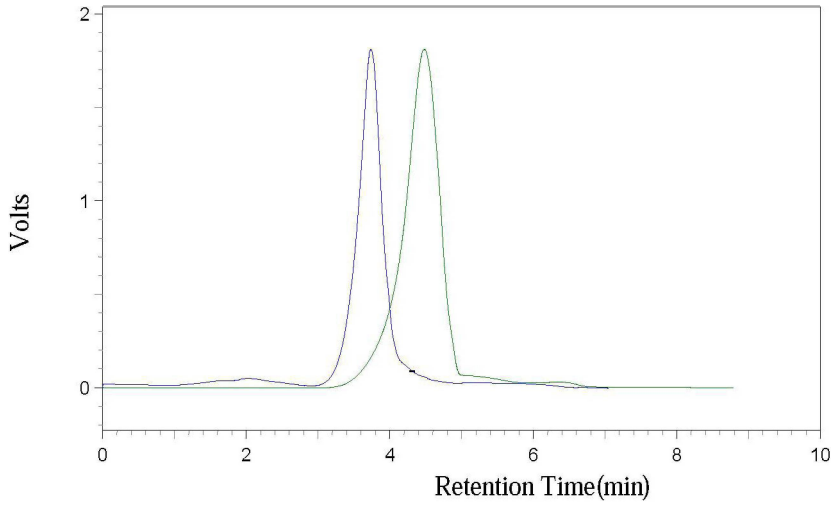


Fig. 1. HPLC chromatograms of TAM and Cold-iodinated-TAM (First peak belongs to TAM and second peak is Cold-iodinated-TAM).

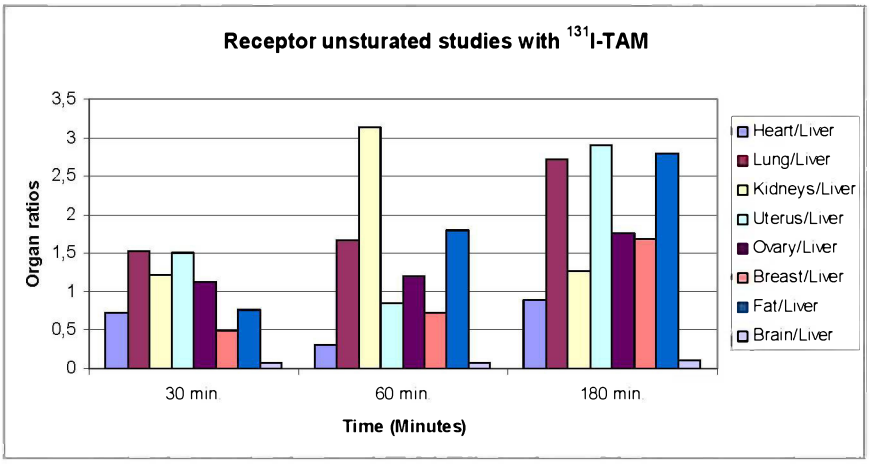


Figure 2. Organ / Liver ratios of receptor unsaturated studies with ^{131}I-TAM for various selected ER-rich tissues.

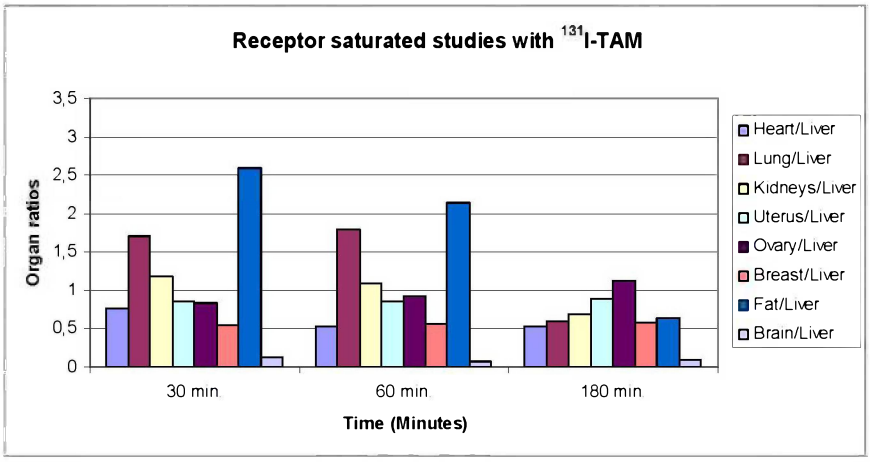


Figure 3. Organ / Liver ratios of receptor saturated studies with ^{131}I-TAM for various selected ER-rich tissues.

Table 1. Migration of component in radio electrophoresis.

	Migration
^{131}I-TAM	Stayed at application point
Na^{131}I	Move to 4 cm