[^{123}I]Idoxifene 합성과 유방암의 세포섭취에 관한 연구

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Study for the Synthesis of [^{123}I]Idoxifene and Its Uptake in the Breast Cancer Cell

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Abstract

Purpose: Idoxifene is currently entering phase Ⅱ clinical trials for the treatment of advanced breast cancer. The radiolabeled idoxifene using ^{123}I provides an opportunity for clinical pharmacology with single photon emission computed tomography (SPECT). The purpose of this study was to prepare radiolabeled idoxifene using ^{123}I and to determine its cell uptake of breast cancer cell line. **Materials and Methods:** With a view to evaluating new anticancer drugs, we are investigating the novel antiestrogen pyrrolidino-4-iodotamoxifen (idoxifene). [^{123}I]Idoxifene has been prepared in no-carrier-added form using a tributyl stannylated precursor which has been synthesized by means of (2-chloroethoxy)benzene with (±)-2-phenylbutanoic acid on the basis of previously reported standard methods. The biodistribution and dynamic behavior of the compound were investigated using the comparative breast cancer cell line, MCF-7 (estrogen receptor-positive) and MDA-MB-468 (non-estrogen receptor). **Results and Conclusion:** Acylation of (2-chloroethoxy)benzene with (±)-2-phenylbutanoic acid gave the versatile ketone (81%) which reacted with 1,4-diiodobenzene to give triphenylethylene as a mixture of E and Z geometric isomers, which were separated by the recrystallization in ethanol. The E-isomer was treated with pyrrolidine to give idoxifene (67%). In order to incorporate radioactive iodine into the 4-position, the 4-stannylated precursor was prepared (30%). The yield of radioiodination was 90-92% with a high radiochemical purity greater than 98%. The ratio of tumor uptake of the breast cancer cell line between MCF-7 and MDA-MB-468 was about 1.7. **(Korean J Nucl Med 2000;34:410-7)**

Key Words: Tamoxifene, [^{123}I]Idoxifene, Antiestrogen, Triphenylethylene, Radioiodine, Stannylated compound

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Introduction

(E)-1-[4-[2-(N-Pyrrolidino)ethoxy]phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene (idoxifene)[1)] is an analogue of the nonsteroidal antiestrogen tamoxi-

fen with an iodine atom in the 4-position and a pyrrolidino ethoxy side chain replacing the diethylamino ethoxy group in the parent compound.

The iodine atom at the 4-position enhances the binding affinity for the estrogen receptor (ER) and also prevents from metabolic 4-hydroxylation.[1,2] Idoxifene has been shown to have a number of potential advantages than tamoxifen for the treatment of breast cancer including enhanced ER binding, improved antagonism of calmodulin-dependent processes,[3,4] reduced partial agonist activity, and enhanced antitumor effect.[3] [^{123}I]-Idoxifene can be used for diagnostic imaging studies with single photon emission computed tomography (SPECT). Because of the low concentration of ERs in tumors, it is necessary to use radioiodinated idoxifene of high specific radioactivity and high radiochemical purity. The tributylstannyl pyrrolidino tamoxifen was considered to be a suitable precursor to introduce radioactive iodine by electrophillic destannylation using chloramine T as an oxidizing agent. The purpose of this study was to synthesize the precursor for radiolabeled compound, to prepare radiolabeled idoxifene using ^{123}I, and to determine the cell uptake of the compound experimentally *in vitro*.

Materials and Methods

1. Chemicals

(2-Chloroethoxy)benzene (>97%) and (±)-2-phenylbutanoic acid (>98%) were purchased from Fluka. Trifluoroacetic anhydride (99+α%), 1,4-diiodobenzene (90%), pyrrolidine (99.5+%), n-butyllithium (1.6 M solution in hexanes), and tri-n-butyltin chloride were purchased from Aldrich. No-carrier-added Na[^{123}I] was obtained from Korea Cancer Center Hospital in reductant free aqueous NaOH. Most of solvents used in synthesis were reagent grade and were distilled immediately before use.

2. Instruments and general procedure

Identification of synthesized compounds was monitored by ^{1}H NMR spectra which were obtained on a Varian Gemini-200 (200 MHz) with $CDCl_3$ solvent. The chemical shifts were reported as the unit of ppm on the basis of tetramethylsilane (Me_4Si). The measurement of melting point for compounds was carried out with Melt-Temp Laboratory Devices Inc., U.S.A. Chromatography indicates flash column chromatography on silica gel (Merck ^{151}1). Radioactivity was measured by radioisotope calibrator, Ramsey NJ 07446 (Model 712M) made by Capintec in U.S.A. Radiochemical purity determinations were performed using silica gel plates (0.25 mm) and Auto Changer 3000, System 200 Imaging Scanner made by Bioscan (Model Sys 200z) from U.S.A. Data aquisition and analysis were carried out by software, Bioscan System 200 Imaging Scanner version 2.247.

All reactions were performed under an inert atmosphere. Purification of E and Z geometric isomers were monitored by ^{1}H NMR spectroscopy and was carried out until none of the unwanted isomer could be detected.

3. Syntheses

Synthetic scheme of idoxifene (3), was shown in Fig. 1. and its synthesis was performed from (2-chloroethoxy)benzene and (±)-2-phenylbutanoic acid as the starting materials.

The procedure was followed essentially that previously reported.[5] Syntheses of precursor for radiolabeling, 4-stannylated idoxifene[6] (4) and radiolabeled [^{123}I]idoxifene[6,7] ([^{123}I]3) were shown in Fig. 2.

4. 1-[4-(2-Chloroethoxy)phenyl]-2-phenyl-1-butanone (1)

In a 250 ml two-neck round flask, (±)-2-phenylbutanoic acid (25.5 g, 156 mmol) and trifluoroacetic anhydride (TFAA) (22.5 ml, 156 mmol) were added and then stirred until the mixture was dissolved completely. To a stirred mixture (2-chloroethoxy)benzene (14.4 g, 12.5 ml, 141 mmol) was added and stirring continued for 16 h under nitrogen gas at room temperature. The mixture was poured into saturated aqueous $NaHCO_3$ (300 ml) and extracted with ethyl acetate (2×300 ml). The combined organic extracts were

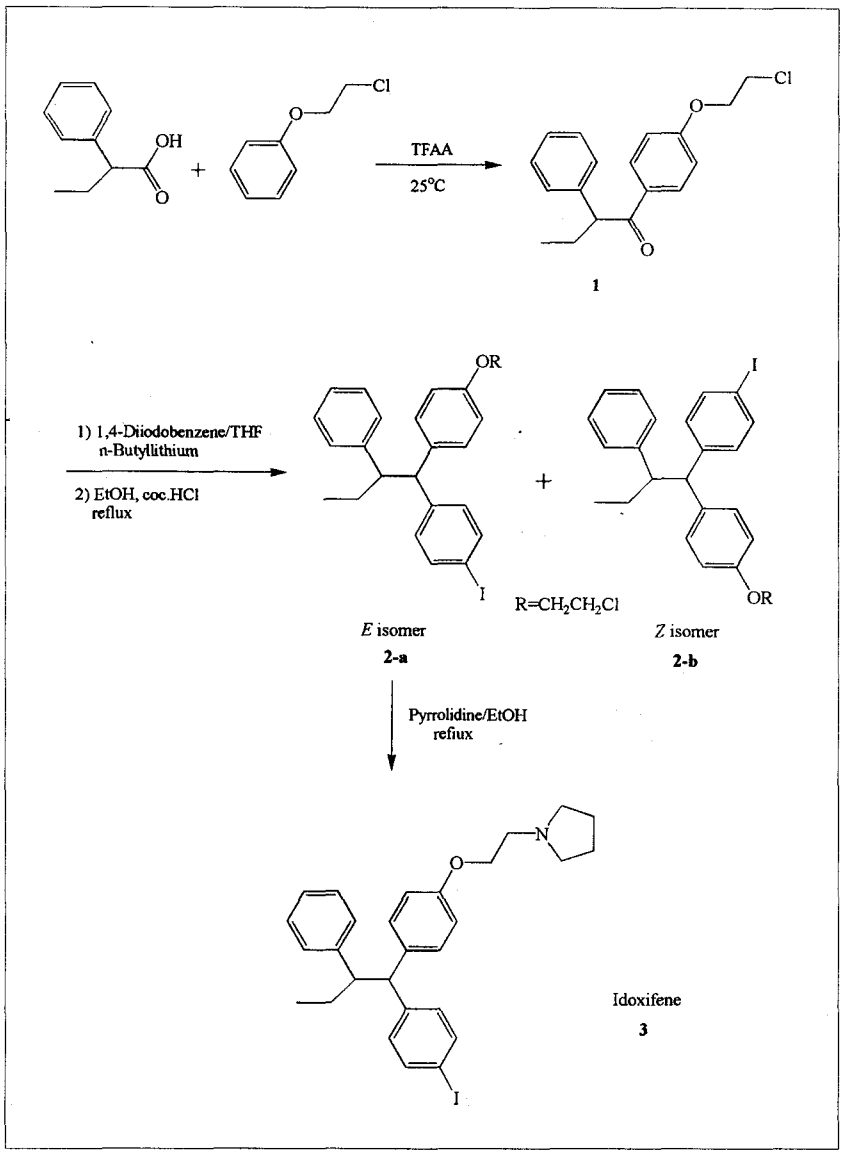

Fig. 1. Synthesis of idoxifene for labeled compound was performed from (2-chloroethoxy)benzene and (±)-2-phenylbutanoic acid as the starting materials.

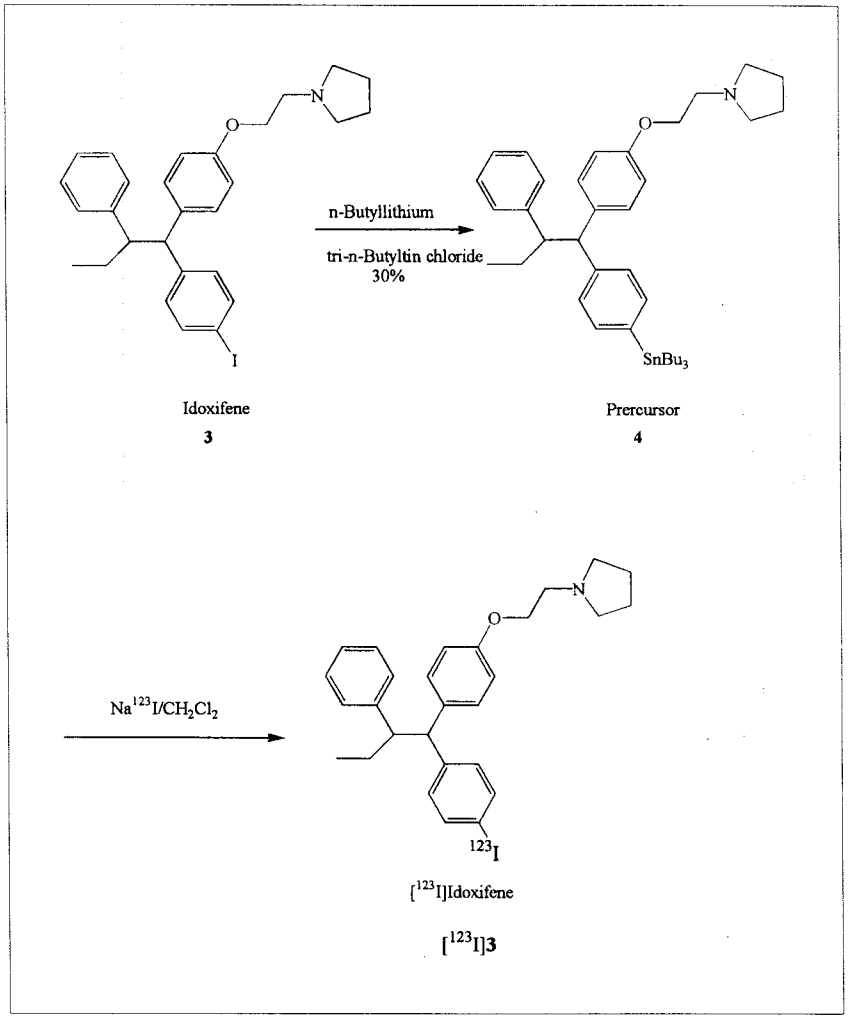

Fig. 2. The synthetic route to prepare radiolabeled [^{123}I]idoxifene from the precursor, stannylated compound.

dried with anhydrous $MgSO_4$ and concentrated in vacuo. The final organic compound was dissolved with ethanol (100 ml) and recrystallized at room temperature to give the white solid (38.14 g, 81%). mp 96~98℃, ^{1}H NMR ($CDCl_3$, δ_H; ppm) 0.90 (t, -CH_3), 1.85~2.18 (d, sept, CH_3CH_2-), 3.80 (t, -CH_2Cl), 4.25(t, -O-CH_2-), 4.44 (t, -CH-), 6.88 (t, 2H, ArH meta to -OCH_2), 7.18~7.22 (m, ^{1}H, ArH para to -CHEt), 7.26~7.34 (m, 3H, ArH ortho and meta to -CHEt), 7.91 (t, 2H, ArH ortho to -OCH_2).

5. (E)-1-[4-(2-Chloroethoxy)phenyl]-2-phenyl-1-(4-iodophenyl)-1-butene (2-a)

In a 250 ml two-neck round flask, 1,4-diiodobenzene (19.2 g, 58.2 mmol) and THF (100 ml) were added and dissolved completely. To a mixture, n-butyllithium (1.6 M. 36.3 ml, 58.2 mmol) in hexanes was added under N_2 at -78℃, and stirring was continued for 1 h. In a reaction vessel the ketone (1) (17.62 g, 58.2 mmol) and THF (50

ml) were added and allowed the mixture at ambient temperature for 24 h. The mixture was poured into ethyl acetate (200 ml) and washed with H_2O (2×100 ml). The organic phase was extracted and dried with $MgSO_4$ and concentrated in vacuo. The residues were dissolved in EtOH (200 ml) and concentrated hydrochloric acid (11.8 M, 100 ml) was added. The mixture was heated at reflux for 5 h and then poured into H_2O (800 ml) and extracted with ethyl acetate (2×150 ml). The combined organic extracts were dried with $MgSO_4$, and concentrated in vacuo. Column chromatography (petroleum ether: CH_2Cl_2=10:1) gave the crude product as a mixture of E and Z geometric isomers. Recrystallization (excess EtOH) gave the E isomer (2-a) as white crystals (2.63 g, 30%). mp 96~97℃ 0.96 (t, 3H, CH_3CH_2), 2.50 (q, 2H, CH_3CH_2), 3.78 (t, 2H, $-CH_2CH_2Cl$), 4.13 (t, 2H, $-CH_2CH_2Cl$), 6.60 (d, 2H, ArH ortho to $-OCH_2$), 7.00 (d, 2H, ArH meta to I), 7.16~7.32 (m, 5H, Ph), 7.71 (ArH ortho to I).

6. (E)-1-[4-(N-Pyrrolidinoethoxy) phenyl]-2-phenyl-1-(4-iodophenyl)-1-butene (3)

In a 100 ml two-neck round flask, chloroethoxy compound (2-a) (1.748 g, 3.76 mmol) and EtOH (35 ml) were added and dissolved by stirring the mixture, and then heated at reflux for 5 h under argon gas. After the product was identified by TLC (petroleum ether:ether:triethylamine=4:4:1), 0.5 M NaOH (30 ml) was added in the reaction vessel. The mixture was concentrated in vacuo. Chromatography (petrol:ether:triethylamine=4:4:1) gave (3) as an off-white solid (1.4 g, 74.5%). mp 108~109℃ ^{1}H NMR ($CDCl_3$, δ H: ppm) 0.95 (t, 3H, $-CH_3$), 1.80 (m, 4H, $N(CH_2CH_2)_2$), 2.48 (q, 2H, CH_3CH_2), 2.45~2.60 (m, 4H, $-N(CH_2CH_2)_2$), 2.87 (t, 2H, $-OCH_2CH_2$), 3.99 (t, 2H, $-OCH_2CH_2$), 6.63 (d, 2H, ArH ortho to $-OCH_2$), 6.76 (d, 2H, ArH meta to $-OCH_2$), 7.06 (d, 2H, ArH meta to I), 7.14~7.32 (m, 4H, Ph), 7.74 (d, 2H, ArH ortho to I).

7. (E)-1-[4-(N-Pyrrolidinoethoxy) phenyl]-2-phenyl-1-[4-(tributylstannyl) phenyl]-2-phenyl-1-butene (4)

(E)-1-[4-(N-Pyrrolidinoethoxy)phenyl]-2-phenyl-1-(4-iodophenyl)-1-butene (3) (0.789 g, 1.56 mmol) and THF (15 ml) were added in an 100 ml two-neck round flask and dissolved completely by stirring with a magnetic bar under an argon atmosphere, and cooled to -78℃ using an acetone/dry ice bath, then n-butyllithium (1.6 M, 1.57 ml, 2.5 mmol) was added. After 10 min, a solution of tributyltin chloride (1.0 ml, 3.69 mmol) in dry tetrahydrofuran (4 ml) was added and the mixture allowed to reach ambient temperature. The product (R_f=0.79) and starting material (R_f=0.60) could be distinguished by thin layer chromatography (petroleum ether:ether:triethylamine=4:4:1). The reaction mixture was poured into ether (50 ml) and aqueous Na_2CO_3 (0.5 M, 50 ml). The organic phase was separated, dried (Na_2SO_4), and concentrated in vacuo. Chromatography on elution with petroleum ether-ether-triethylamine (4:4:1) gave the pure product (4) (0.25 g, 0.463 mmol, 30%) as a colorless oil. ^{1}H NMR ($CDCl_3$, δ H: ppm), 0.89~1.13 (m, 18H, $-(CH_2)3CH_3$ and $EtCH_3$), 1.24~1.64 (m, 12H, $BuCH_2CH_2$), 1.84 (m, 4H, $N(CH_2CH_2)_2$), 2.50 (q, 2H, $EtCH_2$), 2.71 (m, 4H, $N(CH_2CH_2)_2$, 4.04 (t, 2H, $-OCH_2CH_2$), 6.58 (d, 2H, ArH ortho to OR), 6.81 (d, 2H, ArH meta to OR), 7.16~7.30 (m, 5H, ArH), 7.45 (d, 2H, ArH ortho to Sn).

8. Preparation of [^{123}I]idoxifene ([^{123}I]3)

(E)-1-[4-(N-Pyrrolidinoethoxy)phenyl]-2-phenyl-1-[4-(tributylstannyl)phenyl]-2-phenyl-1-butene (4) (precursor, 3 mg, 5.5 μmol) was added in the

reaction vial and dissolved in distilled dichloromethane (500 μl). Na[^{123}I] (250 μl, 1.1 GBq/ml) was added to it, followed by chloramine T solution (88 μM, 500 μl). The reaction was allowed to proceed at room temperature for 30 min, with stirring. The reaction was stopped by addition of 5% $Na_2S_2O_5$ solution (500 μl). The reaction product was extracted in CH_2Cl_2 and the organic phase was separated. Separation of radiolabeled idoxifene from radioiodine and unlabeled precursor was achieved by C-18 Sep-Pak (Millipore Ltd.). Identification of radiolabeled idoxifene was carried out by TLC scanning technique using petroleum ether-ether-triethylamine (4:4:1, v/v%) as eluant (precursor R_f=0.79; radioidoxifene R_f=0.57; free ^{123}I R_f=0.00). Radiochemical yield of 92% were obtained by this method with high radiochemical purity (>98%). Radioidoxifene separated was dried by nitrogen gas and redissolved for i.v. administration by addition of 0.9% NaCl to a final ethanol concentration by 10%.[8]

9. [^{123}I]idoxifene cell uptake

The two cell lines were used to assess [^{123}I]idoxifene uptake. One was MCF-7 (breast adenocarcinoma, ER) and the other was MDA-MB-468 (breast adenocarcinoma, without ER). The culture fluids of cell lines are Eagle's MEM with 1 mM sodium pyruvate (10 μg/ml) as non-essential amino acid, 90% bovine insulin, 10% fetal bovine serum (MCF-7) and 90% Leibowitz's L-15 medium, 10% fetal bovine serum (MDA-MB-468). Two cell lines supplied from American Type Culture Collection (ATCC) were cultured using each culture fluid in 5% CO_2 incubator at 37℃ with exchanging fluids biweekly. Cell lines were taken off in culture flask using 0.25% Trypsin-EDTA, washed with their fluids, 1.0×10^6 clones of each cell line divided in 25 cm^2 culture flask equally, and then cultured for 24 h.

In each flask, [^{123}I]idoxifene of 0.8 μCi/45 μl was added directly. After 5, 10, 30, 60, 120, and 180 min, clones were taken off in each flask, washed with PBS (2×1 ml), and centrifuged (1200 rpm) for 5 min. Upper layer was removed and counted by a γ-counter.

10. Results and discussion

The synthesis of the idoxifene is outlined in Fig. 1. The procedure essentially follows that reported for the synthesis of 4-iodotamoxifen.[5] Acylation of (2-chloroethoxy)benzene with 2-phenylbutanoic acid gave ketone (1) (81%). Reaction of the ketone (1) and (4-iodophenyl)lithium, readily generated by treatment of a diiodobenzene with 1 equiv of n-butyllithium, and subsequent dehydration of the resulting tertiary alcohol gave the triarylbutenes as a mixture of E (2-a) and Z (2-b) geometric isomers, from which it was possible to isolate the E (trans) isomer by fractional crystallization in excess ethanol (30%). The desired E isomers were then treated with pyrrolidine to give the pyrrolidino compound (3) (74.5%). The synthesis of 4-stannylated compound (4) was carried out using pure E idoxifene itself as starting material. Treatment of idoxifene at low temperature with n-butyllithium generated the intermediate 4-lithio compound which was quenched with tributyltin chloride (30%). It is important that the precursor for radioiodination is prepared in an isomerically pure form to avoid having to separate geometric isomers of the labeled compound. E isomer is stereochemically stable. [^{123}I]Idoxifene was synthesized by reaction of 4-stannylated compound (4) and [^{123}I]NaI solution in the presence of the oxidant. The yields of 92% were obtained with high radiochemical purity of about 98% as shown in Fig. 3.

Uptake of [^{123}I]idoxifene at 3 h in MCF-7 with estrogen receptor was higher than MDA-MB-468

(non-ER) as shown in Fig. 4. The ratio of cell uptake percentage MCF-7 in contrast to MDA-MB-468 was about 1.7 at 180 min.

In order to use SPECT for imaging, animal experiments and other clinical applications will be additionally tried.

요 약

목적: 현재 유방암 치료제로서 임상실험 제 2단계에 들어간 idoxifene은 항에스트로겐 의약품으로서 기존의 tamoxifen보다도 많은 장점을 가지고 있는 것으로 연구결과 밝혀졌다. 또한 방사성 동위원소

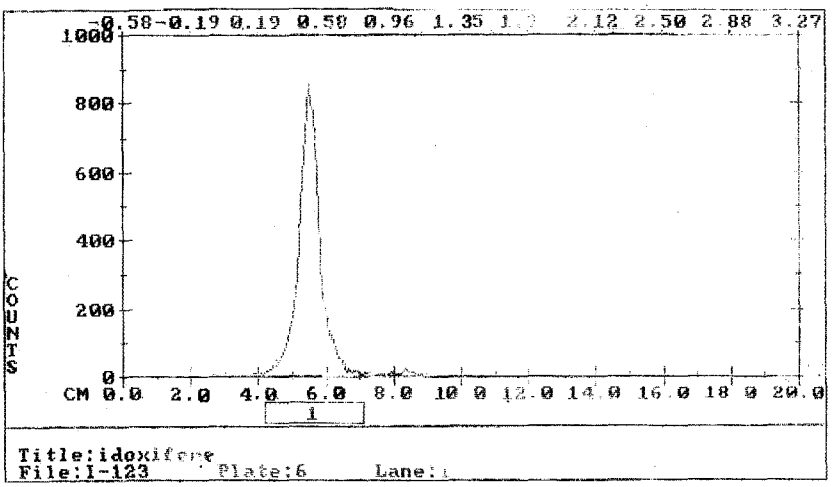

Fig. 3. The TLC chromatogram of [^{123}I]idoxifene is shown as single peak (petroleum ether:ether:triethylamine=4:4:1, Rf=0.57) after separation with Silica Sep-Pak. Labeling yield and radiochemical purity were 92% and 98%, respectively.

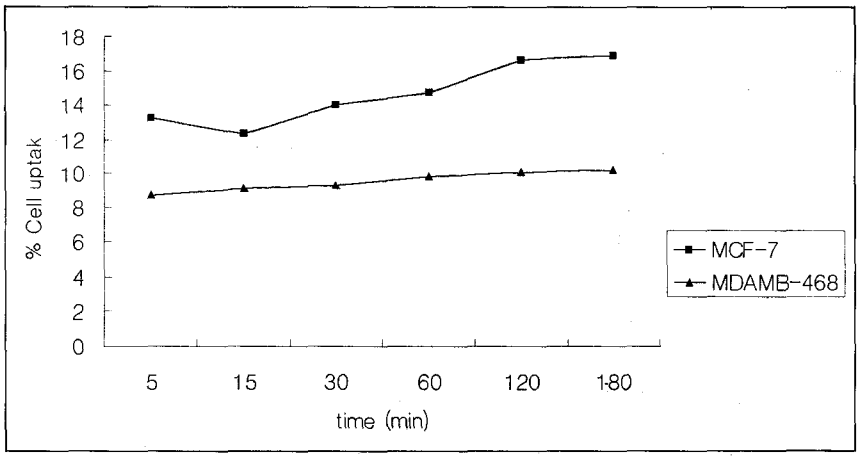

Fig. 4. [^{123}I]Idoxifene % cell uptake of breast tumor cancer with corresponding time interval is indicated. The cell uptake of MCF-7 (ER) was about 1.7 times that of MDA-MB-468 (non-ER) at 180 min.

^{123}I를 표지한 [^{123}I]idoxifene은 SPECT을 이용한 유방암세포를 영상화하여 조기에 진단할 수 있는 진단시약으로서 널리 각광 받고 있다. 따라서 본 연구에서는 idoxifene의 전구체를 합성하고 ^{123}I를 표지하여 세포 내 섭취를 관찰하였다. **대상 및 방법**: [^{123}I]idoxifene을 위한 전구체는 McCague가 연구 발표한 자료를 바탕으로 (2-chloroethoxy)benzene과 2-phenylbutanoic acid를 출발물질로 하여 합성하였다. 표지는 ^{123}I를 사용하였으며 분리는 Silica Sep-Pak을 사용하였으며 세포 내 섭취실험은 에스트로겐 리셉터를 가진 MCF-7과 대조군으로서 에스트로겐 리셉터가 없는 MDA-MB-468을 이용하였다. **결과 및 결론**: Idoxifene의 전구체인 4-stannylated 화합물의 합성수율은 약 30%이었으며, ^{123}I의 표지는 60분 경과에서 90~92%로 최대의 표지수율을 보였으며 방사화학적 순도는 98%이상이었다. 또한 세포 내 섭취실험에서 실험군과 대조군 사이에 섭취비율은 180분에서 1.7:1로 나타나 idoxifene은 항에스트로겐 효과가 아주 높은 것으로 판명되었다. 이를 바탕으로 배양세포와 동물모델을 이용한 추가적인 실험이 필요하며, 유방암 환자에게도 임상이용이 기대된다.

Acknowledgments

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