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journal or publication title	CYRIC annual report
volume	1983
page range	103-107
year	1983
URL	http://hdl.handle.net/10097/49178

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In recent years there has been a great deal of interest in the development of estrogen-receptor-binding radiopharmaceuticals useful for the imaging of estrogen target tissue.¹⁾ Such radiopharmaceuticals could be useful in the scintigraphic detection of breast tumors and their metastases and in the prediction of the response of breast cancer to hormonal therapy.²⁾ Tamoxifen (1), a non-steroid antiestrogen, competes with estradiol for the high affinity cytoplasmic estrogen receptor and binds to this receptor.³⁾ Therefore, we considered it to be of interest to label the tamoxifen with positron emitter⁴⁾ for the detection of a breast tumor. The present paper describes the synthesis of C-11 labeled tamoxifen by the reaction of nortamoxifen (2) with ¹¹CH₃I and the preliminary results of biodistribution in female rats.

Materials and Methods

Tamoxifen citrate was obtained as a gift from ICI Ltd.. The preparation of nortamoxifen was carried out by carbethoxylation of tamoxifen, followed by reductive cleavage with zinc dust⁵⁾ (Fig. 1). High performance liquid chromatography (HPLC) was undertaken using μ -Porasil column. The column was eluted with CHCl₃-MeOH (9:1, v/v), and the eluate was monitored with a UV detector (254 nm) and a sodium iodide scintillation detector.

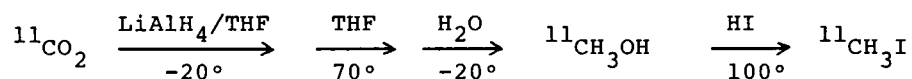
Preparation of 2,2,2-Trichlorocarbethoxynortamoxifen (3).

A stirred mixture of tamoxifen (0.98 g), 2,2,2-trichloroethyl chloroformate (3 ml), anhydrous K₂CO₃ (50 mg) and benzene (60 ml) was refluxed on a oil-bath for 20 hr. After cooling, the reaction mixture was diluted with water and extracted with benzene. The extract was dried and evaporated and the residue was chromatographed on silica gel using CHCl₃ as eluant. The eluate (100 ml) was evaporated to give the carbethoxynortamoxifen (3) (1.05 g, 75.8% yield) as colorless needles, mp 111-112° (from ether-hexane) (Found: C, 62.7; H, 5.20; N, 2.52; C₂₈H₂₈NO₃Cl₃ requires C, 63.1; H, 5.20; N, 2.60%), ¹H-NMR δ (CDCl₃): 0.90 (3H, t, J=7Hz, -CH₂CH₃), 2.46 (2H, quartet, J=7Hz, -CH₂CH₃), 3.06 (3H, s, -NCH₃), 3.63 (2H, t, J=5Hz, -OCH₂CH₂N-), 4.03 (2H, t, J=5Hz, -OCH₂CH₂N-), 4.70 (2H, s, -OCH₂CCl₃), 6.4-7.3 (14H, m, Ar-H). IR_{max}(CHCl₃) cm⁻¹: 1705 (C=O).

Preparation of Nortamoxifen (2). To a solution of carbethoxynortamoxifen (3) (700 mg) in acetic acid (50 ml) was added zinc dust (3 g) by portions with

stirring and refluxing in a oil-bath for 1.5 hr, and then filtered. The filtrate was evaporated, basified with ammonia and extracted with CHCl_3 . The extract was dried and evaporated. The hydrochloride (411 mg, 78.7% yield) afforded colorless crystals, mp 214-216° (from methanol-ether) (Found: C, 75.9; H, 7.11; N, 3.42; $\text{C}_{26}\text{H}_{27}\text{NO}\cdot\text{HCl}$ requires C, 76.2; H, 7.16; N, 3.55%), $^1\text{H-NMR}\delta$ (CDCl_3) (free base): 0.93 (3H, t, $J=7\text{Hz}$, $-\text{CH}_2\text{CH}_3$), 1.80 (1H, s, $-\text{NH}$), 2.46 (3H, s, $-\text{NCH}_3$), 2.47 (2H, quartet, $J=7\text{Hz}$, $-\text{CH}_2\text{CH}_3$), 2.88 (2H, t, $J=5\text{Hz}$, $-\text{OCH}_2\text{CH}_2\text{N}-$), 3.95 (2H, t, $J=5\text{Hz}$, $-\text{OCH}_2\text{CH}_2\text{N}-$), 6.4-7.2 (14H, m, Ar-H). Mass (m/e) : 357 (M^+), 300, 58, 44 (base peak).

Preparation of $^{11}\text{C-CH}_3\text{I}$. The ^{11}C was produced by the nuclear reaction $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ using the Tohoku University Cyclotron and obtained as $^{11}\text{CO}_2$. Carbon-11 labeled CH_3I was prepared from $^{11}\text{CO}_2$ according to the following reaction scheme and supplied by the automated $^{11}\text{CH}_3\text{I}$ synthesis system.⁶⁾ The total time required for the synthesis of $^{11}\text{CH}_3\text{I}$ from $^{11}\text{CO}_2$ was within 25 min.



Synthesis of $^{11}\text{C-Tamoxifen}$. The $^{11}\text{CH}_3\text{I}$ prepared was introduced (flow rate: 200 ml/min ; total time: 5-10 min) into nortamoxifen (from 5 mg of the corresponding hydrochloride) dissolved in acetone (1 ml) at -78° (dry ice-acetone). The reaction mixture was heated at $50-60^\circ$ for 10 min with stirring. After solvent was purged with He gas, the residue was dissolved in CHCl_3 (ca. 700 μl) and the solution was applied onto the HPLC-column. The eluate of labeled tamoxifen was collected and evaporated to dryness. To the residue was added 0.01 N ethanolic HCl (4 ml), then the solvent was evaporated to dryness. The hydrochloride of tamoxifen was dissolved in H_2O (ca. 5 ml) by sonication and sterilized through a membrane filter (0.22 μm). The resulting pH of the solution showed between 5 and 6.

Animal Experiments. Immature female Sprague-Dawley rats (6 weeks) were injected intravenously with the $^{11}\text{C-tamoxifen}$ hydrochloride. At the indicated times, rats were killed by cervical dislocation and tissues were removed, assayed for radioactivity and weighted. The uptake of activity was expressed as per cent of injected dose per g of wet tissue.

Results and Discussion

The reaction product, obtained in 70-80% radiochemical yield, consisted of a mixture of labeled tamoxifen and nortamoxifen. It is important to separate tamoxifen completely from nortamoxifen, since this compound also binds to the estrogen receptor.⁷⁾ The baseline separation between the two compounds could be achieved using a high efficient normal phase HPLC column (Fig. 2). Suboptimal or reverse phase performance (data not shown) that led to tailing of peaks caused contamination of tamoxifen by nortamoxifen. The fraction corresponding to tamoxifen had no other radioactive peaks (Fig. 3) and no impurities were

detectable by ultraviolet absorbance. The mass spectrum of this fraction showed parent ion at m/e 371, and abundant ions at m/e 72 and 58, which⁸⁾ supported this structure (1). These results indicate that labeled tamoxifen is radiochemically and chemically pure (>99.9%). This compound was easily converted to the corresponding hydrochloride suitable for IV injection by the addition of 0.01 N ethanolic HCl. The time required for the synthesis of C-11 tamoxifen hydrochloride was within 40 min from the introducing of ¹¹CH₃I. The total activity and specific activity at the time of use were about 20 mCi and 30 mCi/μmole, respectively.

The data in Fig. 4 show the average tissue concentration of radioactivity following intravenous injection of 4.8 nmole C-11 tamoxifen hydrochloride. Uptake in the uterus was higher than that in the blood. High uptake of radioactivity was also observed in the nontarget tissues (liver, adrenal, kidney and heart). The biochemical characteristics of this ligand to the estrogen-target tissues are now under investigation.

Acknowledgment

This work was partially supported by a Grant-in-Aid for Scientific Research No. 58015010, Ministry of Education, Science and Culture, Japan. We are grateful to ICI Ltd. for a gift of tamoxifen citrate.

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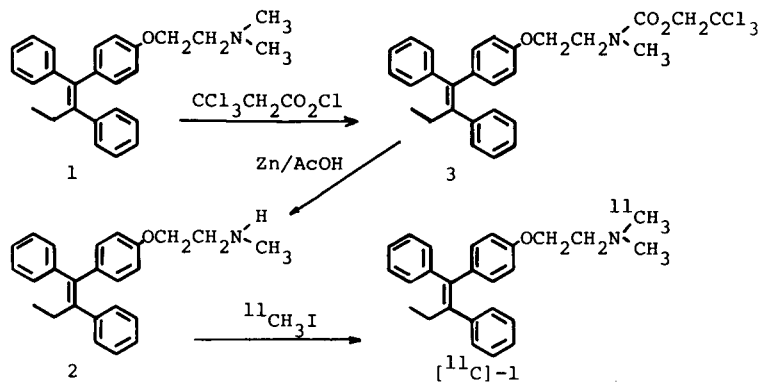


Fig. 1. Synthesis of C-11 tamoxifen.

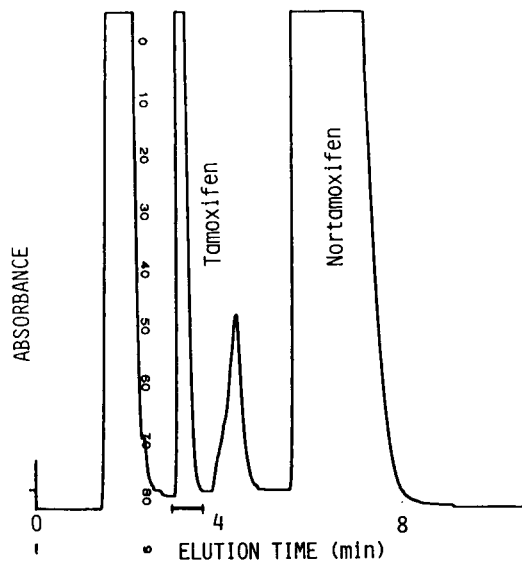


Fig. 2. High performance Liquid Chromatographic Separation of C-11 Tamoxifen from Nortamoxifen.

Bar represents fraction collected.

Analytical conditions

column : μ -Porasil, eluant : CHCl_3 -MeOH (9:1, v/v),

flow rate : 2 ml/min, detector : UV at 254 nm

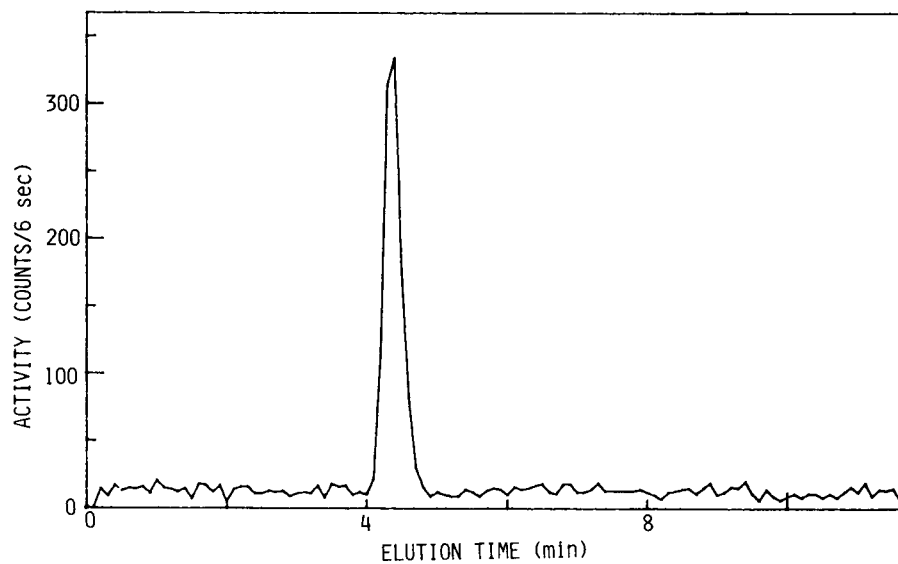


Fig. 3. Radiochemical Analysis of ^{11}C -Tamoxifen with HPLC. Analytical conditions were the same as described in Fig. 2.

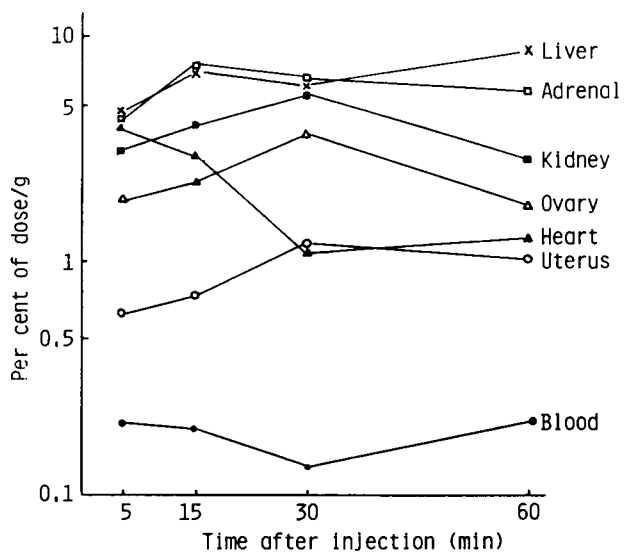


Fig. 4. Tissue concentration of radioactivity following intravenous injection of ^{11}C -tamoxifen hydrochloride.