PRODUCTION OF ^{11}C-LABELED QUINIDINE AND TAMOXIFEN

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SUMMARY

The production of [^{11}C]quinidine and [^{11}C]tamoxifen for use with positron emission tomography is described. The potassium salt of O-desmethylquinidine and N-desmethyltamoxifen free base are treated with [^{11}C]methyl iodide at 130°C for 10 min. After HPLC separation, the [^{11}C-methyl]-labeled compounds are produced with a radiochemical yield of 60 and 70 % respectively. Injectable activities from 50 to 70 mCi are obtained 1 h after the irradiation; the specific activity is 40-60 mCi/μmole. The entire production is controlled by a microprocessor.

Key words : [^{11}C]quinidine, [^{11}C]tamoxifen, [^{11}C]methyl iodide, HPLC.

INTRODUCTION

We have reported (1) a procedure and remote-controlled synthesis system for the production of antipyrine labeled with the short-lived positron emitter ^{11}C for in vivo measurements using positron emission tomography (PET).

The present paper describes a method for labeling the antiarrhithmic quinidine (6'-methoxycinchonine, 1a, Fig. 1) and the non-steroidal antioestrogen tamoxifen (2a) using this system.

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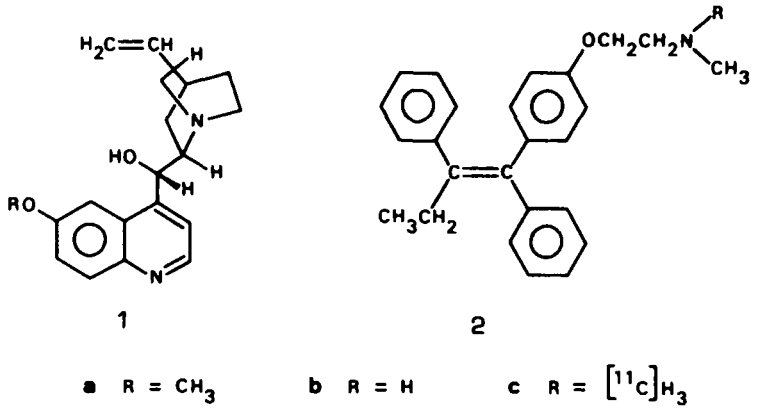
[^{11}C]Methyl iodide serves as labeled precursor of the title compounds, which are formed upon methylation of the corresponding desmethyl homologues.

The synthesis of [^{11}C]quinidine has been developed for use in research concerning the pharmacokinetics and metabolism of the drug. PET studies with [^{11}C]tamoxifen are expected to give additional information about the fate of the drug upon administration to breast-cancer patients (2).



1 2

a R = CH_3 b R = H c R = $[^{11}C]H_3$

FIG. 1. Structure of quinidine (1a) and tamoxifen (2a), of the corresponding desmethyl compounds (b) and of the [^{11}C-methyl]-labeled radiopharmaceuticals (c).

MATERIALS AND METHODS

Synthesized starting materials were purified on a 50 x 0.68 cm HPLC column packed with 10-µm silica (flow rate : 4 ml/min) and the labeled compounds were isolated using a 25 x 1.0 cm column (RSiL 10 µ, Alltech Europe). All HPLC separations were carried out with dichloromethane-methanol-25 % ammonia (v/v) mixtures as eluent, unless stated otherwise.

[^{11}C]Methyl iodide is produced (1,3) from [^{11}C]carbon dioxide, obtained through the $^{14}N(p,\alpha)^{11}C$ nuclear reaction upon irradiation of nitrogen gas in a gas target holder with 18 MeV protons.

A detailed description of the automated and microprocessor-controlled synthesis system can be found elsewhere (4).

[^{11}C]Quinidine.

O-Desmethylquinidine (6'-hydroxycinchonine, 1b), obtained from quinidine (Aldrich) by boron tribromide-mediated ether cleavage in dichloromethane at -75°C, as described by Small et al. (5), was purified with a 70:30:0.2 eluent composition. In a 5 ml cylindroconical reaction vial 0.01 g (0.032 mmoles) of 1b is dissolved in 1 ml of 0.03 M potassium hydroxide in ethanol. The solvent is evaporated at room temperature under a stream of nitrogen and the resulting yellow potassium salt 1 (R = K) is dried under reduced pressure over phosphorus pentoxide for 2 h. The salt is redissolved in 0.25 ml of dry dimethylformamide, giving a yellow-green fluorescing solution.

[^{11}C]Methyl iodide is trapped by bubbling the carrier gas stream (nitrogen) through the solution cooled at -45°C. The mixture is transferred with the aid of a peristaltic pump to a high-pressure reaction vessel (1) and heated at 130°C for 10 min. It is then pumped into a vial connected to the sample loop outlet of the HPLC injection valve and containing 0.002 ml of acetic acid. The reaction vessel and connecting tubing are flushed with 0.75 ml of dichloromethane, used to dilute the sample to a volume of 1 ml.

The solution is injected (6) on the HPLC column and eluted with a 90:9:0.5 solvent mixture at a flow rate of 4.9 ml/min. The elution is monitored by a GM tube and by a UV detector set at 285 nm. The [^{11}C]quinidine peak, which has a retention time of 8 min (Fig. 2,A), is collected in a conically shaped flask, equipped with a magnetic stirrer and an argon gas inlet, preheated to 90°C.

After evaporation of the eluent by applying a stream of argon, the residue is redissolved under stirring in 10 ml of distilled water. The solution containing the ^{11}C-labeled quinidine (1c) is collected in a 20 ml vial with septum cap containing a calculated amount of sodium chloride to obtain an isotonic saline solution for intravenous injection.

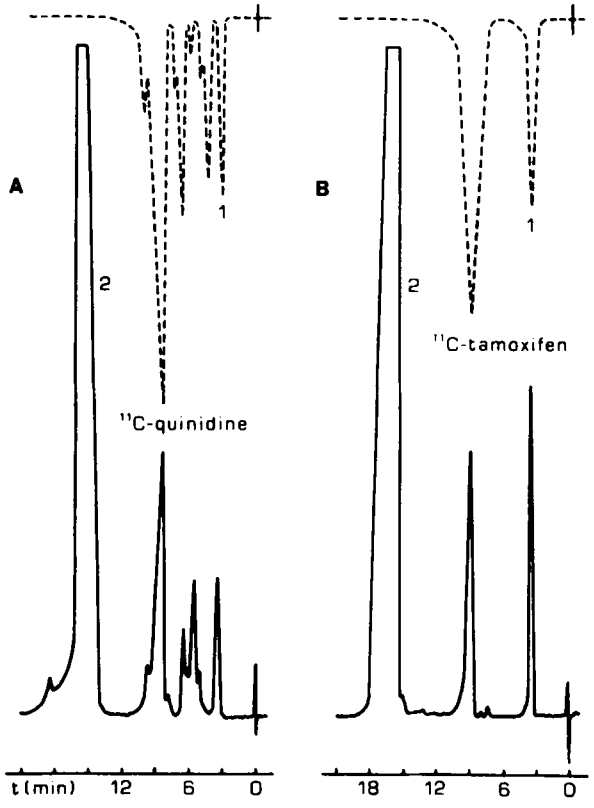


FIG. 2. HPLC chromatograms of [^{11}C]quinidine (A) and [^{11}C]tamoxifen (B). —— = UV trace, --- = radioactivity trace; conditions as described in the text. Peak 1 = [^{11}C]methyl iodide; peak A_2 = O-desmethylquinidine; peak B_2 = N-desmethyltamoxifen.

[^{11}C]Tamoxifen.

N-Desmethyltamoxifen (2b) was obtained upon extraction with dichloromethane of a sodium hydroxide solution of the hydrochloride*. It can also be prepared by N-monodemethylation of tomoxifen (2a) with phenyl chloroformate and subsequent alkaline hydrolysis, according to the method reported by Hobson et al. (7). An additional purification was carried out (composition of the eluent : 98:2:0.1) to remove a small amount of tamoxifen. The reaction mixture for the ^{11}C-synthesis consists of 0.01 g (0.028 mmoles) of 2b in 0.25 ml of dry dimethylformamide.

Essentially the same labeling procedure as described for [^{11}C]quinidine is followed. After the alkylation reaction in the high-pressure vessel at 130°C for 10 min, the reaction mixture is diluted with 0.75 ml of dichloromethane and injected on the HPLC column. The eluent composition is 100:4:0.1, the flow rate 4.9 ml/min and the UV detector is set at 330 nm. [^{11}C]Tamoxifen (2c) is the only radioactive compound present next to some unreacted [^{11}C]methyl iodide and is eluted after 9 min. A typical chromatogram is shown in Fig. 2,B.

To the white solid material left after removal of the eluent is added, under vigorous stirring, 0.1 ml of 40 % citric acid, followed by 0.2 ml of propylene glycol and 9.7 ml of sterile water. The solution is passed over a 0.22-µm Millipore filter and collected in a capped vial. The evaporation flask and connected parts were properly sterilized beforehand.

RESULTS AND DISCUSSION

[^{11}C]Quinidine.

Commercial quinidine is commonly contaminated with 10,11-dihydroquinidine, a dosage form content as high as 20 % being al-

* Gift from ICI Ltd.

lowed by the USP (8). For PET studies its presence under labeled or unlabeled form in the [^{11}C]quinidine preparate is however undesirable. Quinidine and dihydroquinidine are well separated under the HPLC conditions used, but it is still advisable to purify the starting material from demethylated dihydroquinidine as described, because it would consume [^{11}C]methyl iodide.

The addition of base to the mobile phase upon HPLC separation of basic compounds on columns with silica packings is a common procedure (9). A serious drawback however is the dissolution of silica and therefore a limited column life, if the pH of the mobile phase is increased (10). In order to obtain a satisfactory separation between [^{11}C]quinidine and the excess of unreacted O-desmethylquinidine (Fig. 2,A), 0.4-0.5 % of concentrated ammonia has to be added to the eluent. This amount of base slowly dissolves the silica packing of the column, gradually reducing its efficiency and permeability. The life of the HPLC column can however be extended by employing a guard column to saturate the mobile phase with silica (10). The extent of contamination of the labeled material with dissolved silicates, after evaporation of the eluent and redissolution in 10 ml of water, was determined spectrophotometrically using the ammonium molybdate-sulphuric acid method (11). The concentration expressed as silicium dioxide was found to be ca. 0.1 ppm. This is well below the natural blood concentration in man (12); the preparate can therefore be used safely for PET experiments.

The entire synthesis, including HPLC separation and sample preparation, requires 55 min from EOB (end of bombardment). The radiochemical yield based on [^{11}C]methyl iodide is 50-60 %. This is in agreement with the chemical yield of the alkylation reaction (55 % starting from 1 µmole of methyl iodide; determined under

cold conditions), as 1-2 μmoles of methyl iodide carrier are present. In our experimental set-up 100-120 mCi/μA (EOB) of [^{11}C]methyl iodide is produced for an irradiation up to saturation. A 20 min irradiation at a beam intensity of 15 μA yields 450-550 mCi of [^{11}C]methyl iodide, 15 min after EOB, from which 50-60 mCi of [^{11}C]quinidine is obtained 40 min later. The specific activity is then 40-60 mCi/μmole.

The identity of the compound and its purity were checked by HPLC and TLC. Analysis on a silica column under the same conditions as the preparative separation, as well as on a C_{18} reverse-phase column, as described by Kline et al. (13), gave a single peak. UV detection at 235 nm demonstrated the absence of dimethylformamide, the solvent of the alkylation reaction. A TLC plate (Merck Si-60 F-254), developed with ethyl acetate-isopropanol-25 % ammonia 45:35:5 (v/v) as eluent, showed only a single spot when viewed under UV light (254 nm), but revealed a slight contamination using fluorescence detection (excitation wavelength 350 nm) after spraying with 10 % sulphuric acid (14). Upon scanning of the plate, all the radioactivity was found to coincide with the spot of quinidine, indicating the satisfactory radiochemical purity of the compound.

[^{11}C]Tamoxifen.

The separation of [^{11}C]tamoxifen from the excess of starting material is adequate using an eluent with an ammonia content of 0.1 % (Fig. 2,B). At this base concentration the dissolution of silica is negligible, as demonstrated by the long column life. The use of a guard column is therefore unnecessary in this case.

A special problem presents the low solubility of tamoxifen in water due to its apolar nature. In order to obtain a solution for intravenous injection, the solubility of tamoxifen in different

aqueous solutions was investigated. Being a weak base, the solubility in water is improved by neutralisation with an excess of acid. Since the order of tamoxifen salt solubility is chloride < acetate < citrate, citric acid was chosen for the neutralisation. Fig. 3 shows the experimental solubility curve of tamoxifen (line A) in function of the citric acid concentration. The solutions were made isotonic by addition of an appropriate amount of propylene glycol, that also serves as a cosolvent. Above line B tamoxifen solutions precipitate by dilution with isotonic phosphate buffer of pH 7.4. The area below line B represents the solutions which do not precipitate by dilution.

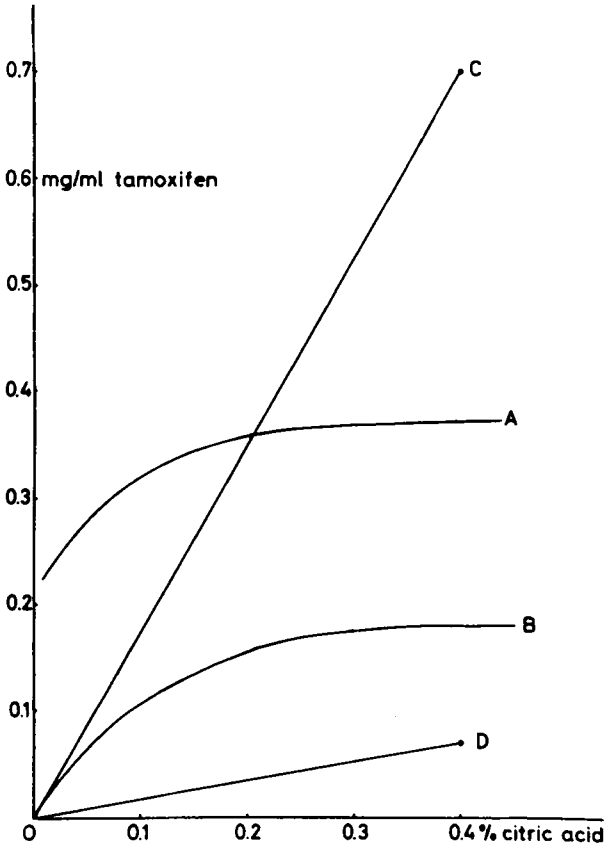


FIG. 3. Solubility chart of tamoxifen in aqueous citric acid solutions. See text for explanation.

It is important to realize that when a solubilized drug solution is injected intravenously, both the drug and the cosolvents are diluted at the same rate. Fig. 3 also shows two theoretical dilution curves of tamoxifen solutions of respectively 0.7 mg/ml (line C) and 0.07 mg/ml (line D) in 0.4 % citric acid, made isotonic with propylene glycol. The parts of line C which are situated above line A and between line A and line B represent the areas of potential precipitation. Line D lies entirely below line B. In the latter situation there is no possibility of tamoxifen precipitation. The amount of tamoxifen carrier in a production is about 0.3-0.5 mg (determined by HPLC using standard solutions). To guarantee that tamoxifen is entirely in the citrate form, 0.1 ml of 40 % citric acid is added at 90°C, followed after a few seconds by 0.2 ml of propylene glycol and 9.7 ml of water. Thus the final tamoxifen concentration is 0.03-0.05 mg/ml in 10 ml of 0.4 % citric acid. This solution is even less concentrated than the start solution of line D (Fig. 3), so that there is no possibility of precipitation by dilution with isotonic phosphate buffer. Neither temperature differences between the injection fluid and the body, nor the difference between blood and isotonic phosphate buffer are taken into account. Preliminary animal experiments indicated that tamoxifen, solubilized as proposed, does not precipitate after injection.

The method for HPLC purification and sample preparation of [^{11}C]tamoxifen described is an improvement compared to the procedure reported by Svärd et al. (15), whereby the compound is obtained in a water-ethanol-acetic acid solution, which could lead to precipitation upon intravenous injection.

The production of [^{11}C]tamoxifen requires 60 min from EOB. The chemical yield, as determined under cold conditions, varies

from 60 % for 1 µmole of methyl iodide to 90 % for 4 µmoles. This agrees with the radiochemical yield of 60-70 % (70 mCi/µA at EOB for an irradiation to saturation), taking the amount of methyl iodide carrier (1-2 µmoles) into account. In routine production (irradiation time : 20 min; beam intensity : 15 µA) 60-70 mCi of [^{11}C]tamoxifen is obtained, with a specific activity of 40-60 mCi/µmole.

By TLC and HPLC analysis, the compound was found to be chemically and radiochemically pure. The ^{1}H NMR spectrum (16) recorded of the combined materials of three productions showed that no isomerisation to the corresponding cis triarylethylene occurs during the methylation reaction. Tests proved the sterility and apyrogenicity of the [^{11}C]tamoxifen solution for injection.

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