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Note

Synthesis of [^{125}I]amiodarone and its purification by high-performance liquid chromatography

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Amiodarone, an anti-arrhythmic drug, is widely used[1]. The details of its metabolism are still obscure, although several metabolites have been described[2]. The production of an excess of molecular iodine induced by this iodine-containing drug could explain the adverse effects on the thyroid function observed during amiodarone therapy[3].

This deiodination of amiodarone cannot be investigated by measurement of the stable isotope ^{127}I. Since there are no reliable methods of separating and measuring small amounts of iodide in the presence of both amiodarone and iodinated metabolites. Therefore there is a need for isotopic labelling methods[4].

The aims of this work are to present new methods for (1) the preparation of [^{125}I]amiodarone, (2) the purification of labelled amiodarone and (3) the control of its radiochemical purity.

METHODS AND RESULTS

Preparation of [^{125}I]amiodarone

Labelling of 2-butyl-3-(3,5-diiodo-4-hydroxybenzoyl)benzofuran. This compound (S1086) used in the industrial synthesis of amiodarone[4] has a diiodinated phenol group which can easily be radiolabelled. ^{125}I was chosen for its long half-life. The oxidation of ^{125}I- (0.1–1 mCi) was achieved by the Chloramine T method. An exchange reaction (^{125}I → ^{127}I) takes place in S 1086.

One milligram of S 1086 was dissolved in 1 ml of absolute methanol then 9 ml of 0.1 *M* phosphate buffer pH 7.4 were added. 0.1 ml of this solution (10 μg of S 1086 or $1.83 \cdot 10^{-8}$ mole) was used for the labelling, which otherwise was done according to usual methods[5].

Purification of [^{125}I]S 1086 and control of labelling yield. Following labelling, [^{125}I]S 1086 was immediately separated from unreacted ^{125}I$^-$ by chromatography on

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a column (0.5 × 3 cm) of anionic resin AG 1X2 (200–400 mesh). The reaction mixture was applied to the column, first equilibrated with absolute methanol (which has no apparent adverse effect on the resin). The column was washed three times with 5 ml of methanol. Then [^{125}I]S 1086 was eluted with 2 × 5 ml of 17 *M* acetic acid–methanol (10:90 v/v). Unreacted $^{125}I^-$ was almost totally retained on the resin. The effluent was vacuum-desiccated, the residue dissolved in methanol and dried three times to get rid of any acetic acid. The labelling yield was found to be 80–90%, calculated as the ratio of the radioactivity recovered in the effluent to that applied to the column.

Synthesis of [^{125}I]amiodarone. The desiccated [^{125}I]S 1086 was dissolved in 1 ml of benzene and 0.1 ml of an aqueous solution of 0.4 *M* Na_2CO_3 was added to ensure that the solution remained alkaline. The mixture was stirred for 1 min then heated at 85°C in a water-bath for 30 min. A coupling reaction was then carried out with an excess of diethylaminoethane chloride, hydrochloride (S 1088) as 0.1 ml of a 0.2 *M* aqueous solution ($2 \cdot 10^{-5}$ mole). The molar ratio of the two reagents S 1088/S 1086 was *ca.* 1000.

The mixture was stirred then heated (85°C) for 10 min. After cooling (25°C), then synthesized [^{125}I]amiodarone was extracted with 3 × 1 ml of diethyl ether. The extracts were pooled and washed three times with water. The ether was then vacuum desiccated and the [^{125}I]amiodarone dissolved in methanol.

Prepurification and control of [^{125}I]amiodarone synthesis. Prepurification was achieved by anionic resin column chromatography as described above. [^{125}I]Amiodarone, which is not retained on AG 1X2, was recovered in methanol. The yield of the synthesis was checked and the solution was then concentrated.

About 80% of the radioactivity was recovered in the methanol fraction as crude amiodarone. The unreacted [^{125}I]S 1086 could be eluted from the column with acetic acid–methanol (10: 90 v/v) to measure the yield of the coupling reaction. $^{125}I^-$ is again retained by the resin.

Purification by high-performance liquid chromatography (HPLC)

Diethyl ether extraction or AG 1X2 chromatography of the crude preparation were not found sufficient to obtain pure [^{125}I]amiodarone.

A Lirec Model A 802 constant flow chromatography pump connected via a 50-μl loop to a Rheodyne injector comprised the solvent delivery system. A Resolve TM column (150 × 3.9 mm; Waters Assoc., Milford, MA, U.S.A.) was packed with 5-μm spherical silica. Chromatographic purification of [^{125}I]amiodarone was carried out with methanol–ammonia (999.9:0.1 v/v) pumped at a constant flow-rate of 0.5 ml/min, the pressure being 20 bars (280 p.s.i.).

During the chromatography, up to 70 fractions of 0.08 ml were collected using a Gilson Microcol TDC 80 fraction collector. The chromatographic separation was standardized with unlabelled amiodarone and S 1086 monitored by a UV spectrophotometer at a wavelength of 243 nm. The fractions collected were analysed for radioactivity with an automatic gamma sample changer (efficiency: $1 \cdot 10^6$ cpm/μCi or $3.7 \cdot 10^4$ Becquerel).

The radioactivity profile and the corresponding UV absorption of the fractions displayed two major peaks (Fig. 1). The retention time and profile of the second peak were identical to those of pure amiodarone. The retention time of amiodarone was

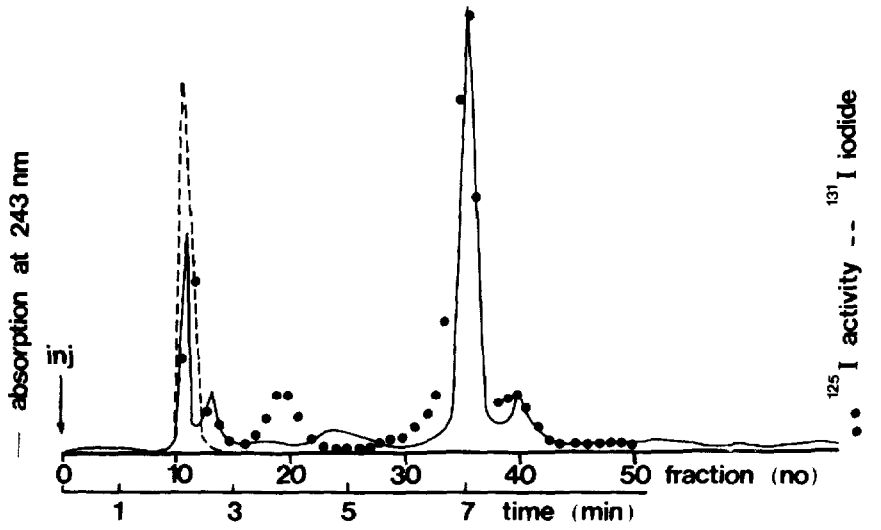

Fig. 1. UV absorption (—) and radioactivity (●) profiles of [^{125}I]amiodarone during HPLC purification. ^{131}I was added as control (- - -).

found to be 7 min (k' = 2.5). In the first peak were found chemical as well as radioactive impurities (mainly $^{125}I^-$ and [^{125}I]S 1086). The retention time was about 3 min. No overlap was observed between the two peaks. After the first HPLC purification, [^{125}I]amiodarone was found to be only partially purified, thus requiring further HPLC (Fig. 2).

Control of purity

The identification of the labelled molecule thus obtained was achieved by successive crystallizations with [^{127}I]amiodarone to constant specific acitivity. This painstaking method remains the most efficient, sensitive and convincing test of radiochemical purity.

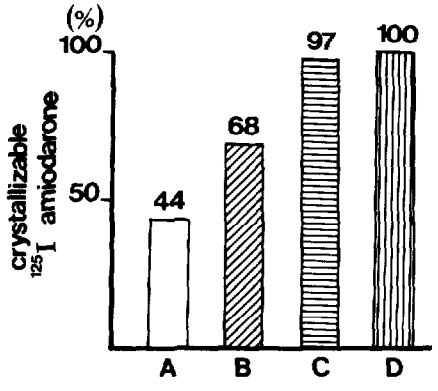

Fig. 2. Purity of [^{125}I]amiodarone as determined by crystallizations to constant specific activity. A, Crude (major amiodarone HPLC peak removed); B, crude preparation; C, once HPLC purified; D, twice HPLC purified.

TABLE I

TEST OF PURITY OF [^{125}I]AMIODARONE BY SUCCESSIVE CRYSTALLIZATIONS TO CONSTANT SPECIFIC ACTIVITY

Preparation	*Property*	*Before crystallization*	*No. of crystallizations*			
			1	*2*	*3*	*4*
Crude	Weight of [^{127}I]amiodarone crystals (mg)	165.95	135.05	114.50	95.05	63.00
	Total radioactivity of crystals (cpm)	94,005*	57,654	46,025	37,105	24,227
	Crystallizable [^{125}I]amiodarone (%)	–	75.4	71.0	68.9	67.9
Twice HPLC purified	Weight of [^{127}I]amiodarone crystals (mg)	243.75	216.60	201.50	178.35	160.50
	Total radioactivity of crystals (cpm)	97,196*	87,383	80,629	71,714	64,216
	Crystallizable [^{125}I]amiodarone (%)	–	101.2	100.3	100.9	100.1

* ^{125}I radioactivity of the preparation (to be tested for its [^{125}I]amiodarone content) added to the [^{127}I]amiodarone solution before the first crystallization.

From 0.05 to 0.1 μCi of the [^{125}I]amiodarone preparation were added to 150–200 mg of [^{127}I]amiodarone in the form of a pure crystallized powder. The amiodarone was dissolved by adding 1 ml of absolute ethanol, then heating at 70°C in a water-bath for a few seconds. After the addition of 2 ml of diethyl ether, crystallization occurred upon cooling for a few hours, with a crystallization yield (by weight) of 80–90%.

The crystals were washed twice with ethanol–diethyl ether (1:2 v/v) and once with ether alone. They were then desiccated to constant weight in an oven at 70°C. The radioactivity of a weighed amount of the crystals was measured, corrections for autoabsorption and geometry being made. Examples of tests of purity are given in Table I.

Fig. 2 shows the HPLC purification of [^{125}I]amiodarone. As shown by crystallizations to constant specific activity, amiodarone was found to be 97% pure after one HPLC purification and after two such purification steps, radioactive amiodarone co-crystallized quantitatively with [^{127}I]amiodarone. The radiochemical purity was found to be higher than 99%. No impurity detectable by crystallization was found to occur when a methanolic solution was stored at -20°C for least 1 month.

DISCUSSION

The labelled amiodarone preparation obtained is characterized by its high specific activity (50 μCi/mmole), high radiochemical purity and freedom from iodide contamination. A higher specific activity could probably have been obtained but was not sought.

The proposed method of preparation and purification of labelled amiodarone has been made as simple and reproducible as possible so that it can be repeated by any other laboratory. Other methods are undoubtedly possible. However, we never succeeded in obtaining direct labelling of amiodarone by simple iodine exchange reactions, a fact which might be attributed to the substituted phenol group of amiodarone.

This method of purifying amiodarone is based on the known solubility of amiodarone in organic solvents (alcohols, ethers, etc.) as opposed to its almost complete insolubility in water —at least in the absence of proteins and other water-soluble biological substances. It should be emphasized that the method could not be applied as such to amiodarone-containing biological fluids. For this purpose, recent studies[6,7] of amiodarone and amiodarone metabolites made use of a preliminary extraction. Therefore, the compounds which remain, after extraction, in the aqueous fraction, have been ignored. It remains to be seen whether they are iodide or water-soluble metabolites of amiodarone.

The question as to whether amiodarone is deiodinated *in vivo* is unresolved. Thus far no method has been found capable of separating stable iodide (from deiodination) and amiodarone. An earlier study[4] made use of [^{131}I]amiodarone of very low specific activity and suggested that some deiodination takes place. However, the purity of this labelled molecule was not stated.

The reported method includes two steps, anion-exchange resin chromatography and HPLC, which combine efficiently to remove any detectable trace of I^- from the labelled compound. The labelled amiodarone thus prepared is especially suitable for investigating amiodarone deiodination.

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