Journal of Chromatography, 374 (1986) 394—399
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

CHROMBIO. 2860

Note

Analysis of amiodarone and desethylamiodarone in serum and tears by reversed-phase high-performance liquid chromatography

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(First received January 4th, 1985; revised manuscript received September 9th, 1985)

Amiodarone is a potent antiarrhythmic agent used in the treatment of refractory tachyarrhythmias and premature ventricular contractions [1]. The recent development of sensitive and selective analytical procedures for this drug in serum has helped to elucidate some of its distribution and elimination characteristics [2—6]. However, these studies have yet to define a range of serum drug concentrations which are associated with maximal therapeutic effects and minimal undesirable side-effects [3]. Dosages of amiodarone have therefore been chosen on an empirical basis and this may be responsible for the frequent and numerous side-effects which have been reported with this drug [7]. In particular, nearly all adults on long-term therapy develop corneal microdeposits, the appearance of which is related to the duration of therapy (onset occurring at four to sixteen weeks) and up to 10% of patients eventually complain of glare and colored haloes around point light sources [8]. These deposits and symptoms appear to resolve slowly on discontinuation of therapy. Although direct drug action presumably induces this cytopathology, the mechanism of drug access to the corneal epithelium is unknown. Amiodarone and/or its de-ethylated metabolite may be secreted in the lachrymal fluid or may diffuse through conjunctival and limbal vessels. The former is supported, but not confirmed, by clinical observation and animal research [9]. According-

0378-4347/86/$03.50

ly, we have developed a reversed-phase high-performance liquid chromatographic (HPLC) analysis for amiodarone and its metabolite in human serum and tears in an attempt to test this hypothesis.

EXPERIMENTAL

Instrumentation

A Model 110A high-pressure solvent delivery pump was used in conjunction with a Model 210 manual injection valve and a 155-10 variable-wavelength UV detector (Altex, Berkeley, CA, U.S.A.). Separation and quantitation were achieved on an Ultrasphere octyl 5-μm (15 cm × 4.6 mm) analytical column (Altex). Detector wavelength was set at 240 nm (the absorbance maximum for amiodarone) in order to maximize sensitivity. Sensitivity was approximately halved at 254 nm.

Chromatographic conditions

A flow-rate of 2.0 ml/min was used at ambient temperature generating a back-pressure of approximately 100 bar (1500 p.s.i.). Under these conditions, the retention times of trifluoperazine, metabolite and amiodarone were 5.4, 6.4 and 7.2 min, respectively.

Reagents and standards

Anhydrous amiodarone hydrochloride and N-desethylamiodarone hydrochloride were obtained from Labaz (Brussels, Belgium). The internal standard, anhydrous trifluoperazine dihydrochloride, was obtained from Smith, Kline and French (Philadelphia, PA, U.S.A.) and was chosen because this compound is easily available, chemically similar (a lipophillic base) and displayed similar UV absorbance, solvent extraction and reversed-phase HPLC chracteristics to amiodarone. Stock solutions of approximately 100 and 10 mg/l of both compounds were prepared in methanol. All buffer salts were reagent grade, hexane was nanograde and methanol and acetonitrile were HPLC grade. The HPLC mobile phase consisted of 0.01 *M* ammonium acetate, pH 4.0 (10%) and a 50:50 (v/v) mixture of methanol—acetonitrile (90%).

Procedure

Serum. To a 15-ml disposable screw-capped culture tube were added 1.0 ml serum, 20 μl internal standard solution (100 mg/l) and 0.5 ml sodium acetate (1.0 *M*, pH 5.5) buffer. The sample was extracted with 5 ml hexane by gentle shaking (to prevent the formation of an emulsion) for 30 min. The organic phase was separated by centrifugation at 1500 *g* for 20 min and transferred to a clean glass disposable culture tube. The sample was evaporated to dryness under nitrogen and was made up into 250 μl methanol of which 25 μl were injected onto the analytical system. Standard curves of amiodarone and metabolite in serum were prepared by pipetting an appropriate volume of the standard solutions into the 15-ml culture tube prior to the sample preparation procedure described above.

Tears. The procedure for tears was essentially the same as that for serum with the exception that only 100 μl of tear sample was used and 10 μl of the

dilute internal standard solution (10 mg/l) were added. The evaporated sample was made up into 100 μl of methanol of which 75 μl were injected onto the HPLC apparatus. Standard curves of amiodarone and metabolite in tears were prepared by pipetting an appropriate volume of the standard solutions into the 15-ml culture tube prior to the sample preparation procedure described above.

RESULTS

The results of the injection of a standard solution of amiodarone, N-desethylamiodarone and trifluoperazine onto the analytical system are shown in Fig. 1. The chromatograms from the serum and tears of an individual not receiving amiodarone or any other medication is shown in Fig. 2. It can be seen that there are no endogenous interfering peaks at or after the retention time of the parent drug or metabolite. Fig. 3 shows the results of analysis of the serum and tears from a patient receiving chronic amiodarone therapy at a dose of 400 mg per day. The overall recoveries of amiodarone, N-desethyl-

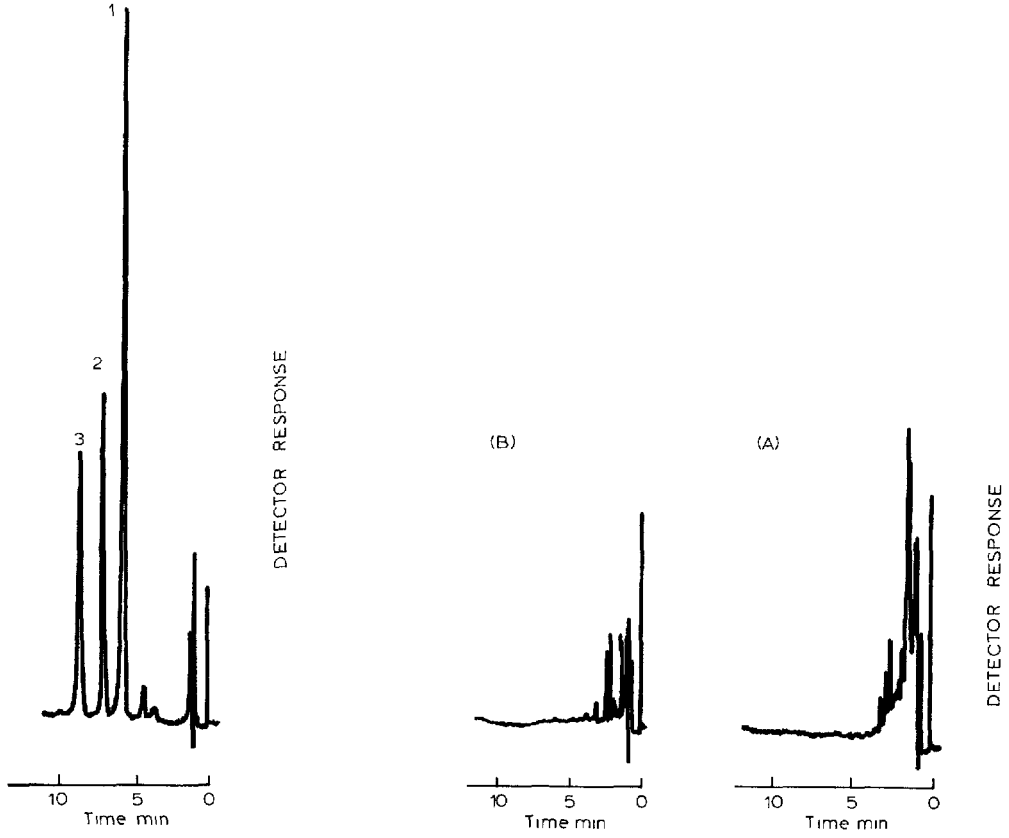

Fig. 1. Injection of a methanolic standard solution of trifluoperazine (1), N-desethylamiodarone (2) and amiodarone (3) onto the HPLC system. The vertical axis scale is absorbance (0.02 a.u.f.s.).

Fig. 2. Injection of blank serum (A) and blank tear (B) sample onto the HPLC system after preparing the samples as described in the text. The vertical axis scale is absorbance (0.02 a.u.f.s.).

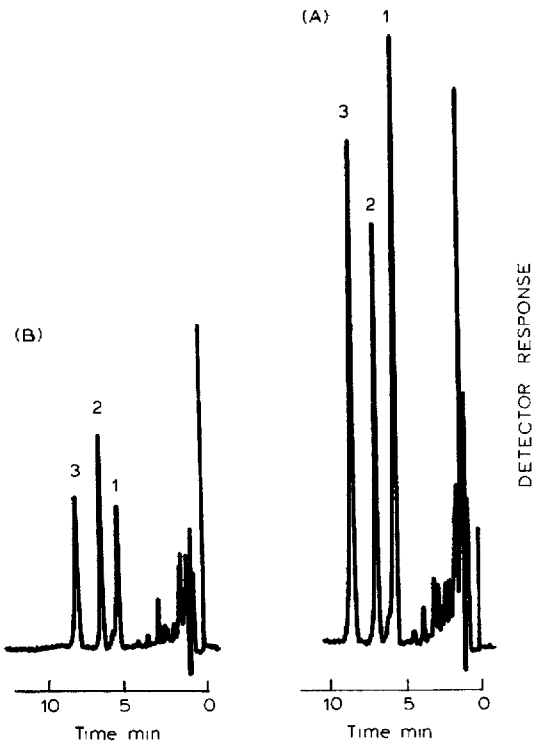

Fig. 3. Injection of a serum (A) and tear (B) sample from a patient receiving chronic amiodarone therapy at a dose of 400 mg per day. The vertical axis scale is absorbance (0.02 a.u.f.s.). Peaks: 1 = trifluoperazine; 2 = N-desethylamiodarone; 3 = amiodarone.

amiodarone and trifluoperazine from serum were 94, 92 and 74%, respectively, and from tears 97, 96 and 80%, respectively. Quantitation of amiodarone or N-desethylamiodarone was achieved by comparing their respective peak-height ratios to trifluoperazine in patient samples to those of known samples of the standard curve. The inter-day coefficients of variation (C.V.) in the quantitation of control samples of 1000 and 200 ng/ml were 7.1 and 10.2%, respectively, for amiodarone and 5.1 and 8.9%, respectively, for N-desethylamiodarone. The intra-day C.V. for the same controls were 4.5 and 5.3%, respectively, for amiodarone and 5.1 and 4.9%, respectively, for N-desethylamiodarone. The standard curves for both parent drug and metabolite were linear over the range of 50 ng/ml to 10 μg/ml and the sensitivity (three times baseline noise) was approximately 20 ng/ml for both compounds in human plasma and tears.

DISCUSSION

Previously published assays for amiodarone in biological fluids and tissues possess characteristics which may limit their general implementation and utility in many research laboratories. These characteristics include the lack of an internal standard [3], the use of an internal standard which may not be generally available [2, 4, 5], or which has chromatographic characteristics quite dissimilar to amiodarone [6], the use of unstable or highly volatile extraction

or mobile phase solvents [2, 5], the need for gradient flow programming [5], the inability to detect the metabolite of amiodarone [3—6] or the use of ammonia-based solvent systems with bonded phase columns [13]. Some authors [2, 4, 10] have advocated the use of normal-phase chromatography (silica gel columns with non-aqueous solvents) for basic compounds in order to minimize mixed-mode separation and the resultant peak tailing and excessive retention seen in some reversed-phase assays. However, these problems can be minimized in many cases by the inclusion of a competing base in the mobile phase of the reversed-phase chromatographic eluent [12]. The presence of a competing base in the mobile phase (usually an alkylamine derivative but in this instance ammonium) appears to minimize binding to exposed silica with resultant poor peak symmetry and excessive retention. These observations are consistent with current concepts on mixed-mode separation of basic compounds on C_{18} bonded phase columns.

The concentration of N-desethylamiodarone was approximately equal to that of amiodarone in serum from patients receiving the drug on a chronic basis but was approximately three-fold that of amiodarone in the tears of these patients, indicating differential distribution characteristic of parent drug and metabolite into tears. The serum-to-tear concentration ratios for amiodarone and metabolite were approximately 10 and 3, respectively, indicating that both compounds were not entirely free to distribute homogeneously between these fluids (perhaps due to differences in plasma protein binding or pH partition characteristics). However, this relationship was much more variable.

The results of the present assay indicate that it can be used for the routine monitoring of amiodarone and N-desethylamiodarone in the serum and tears of patients receiving the drug on a chronic basis. Additionally it posesses the necessary sensitivity, selectivity and reproducibility to be used in clinical pharmacokinetic and toxicologic studies which may provide further insight into the mechanism of action and toxicity of this drug in patients.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Dr. B.N. Singh for permission to study patients under his care.

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