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Determination of the class III antiarrhythmic drugs dronedarone and amiodarone, and their principal metabolites in plasma and myocardium by high-performance liquid chromatography and UV-detection

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ABSTRACT

Dronedarone, a noniodinated benzofuran derivative of amiodarone, is believed to have a better side effect profile, and is currently undergoing phase III clinical trials. A novel method was developed for the determination of dronedarone and its principal metabolite debutyldronedarone in both plasma and myocardial tissue by high-performance liquid chromatography (HPLC) coupled with UV-detection. The assay was also validated for determination of amiodarone and desethylamiodarone. Samples were obtained from healthy humans (plasma) and goats (plasma and myocardium). Sample preparation included deproteinization with acetonitrile and extraction with a mixture of heptane and dichloromethane (50/50, v/v). Chromatographic separation was performed on a Pathfinder PS polymeric C18 column (50 mm × 4.6 mm, 2.5 μm) with a mobile phase of acetonitrile, isopropanol, water and ammonia (80/10/10/0.025, v/v/v/v) at a flow-rate of 1 ml/min. Calibration curves of all analytes were linear in the range of 0.01–5 μg/ml for plasma samples, with a lower limit of quantification (LLOQ) of 0.04 μg/ml. For myocardial tissue samples, linear curves of all analytes were observed in the range of 0.02–500 μg/g, with a LLOQ of 0.08 μg/g. Within- and between-day precision was <18%, and within- and between-day accuracy ranged from 97.5 to 109.7%, with a recovery of 67.6–79.9%. The present method enables sensitive and specific detection of dronedarone, amiodarone and principal metabolites in plasma as well as myocardial tissue.

1. Introduction

Amiodarone, a benzofuran derivative synthesized in 1962 as a coronary vasodilator, is now recognized as one of the most effective drugs in the treatment of both atrial and ventricular arrhythmias [1]. It exerts very complex electropharmacologic effects, including classes I, II, III and IV antiarrhythmic actions, with marked differences between short-term and long-term effects [2]. Despite its potent efficacy, long-term use of amiodarone is limited by serious side effects such as thyroid dysfunction, pulmonary toxicity and hepatic toxicity [3]. Unusual pharmacokinetic characteristics, especially the large volume of distribution and long elimination half-life (up to 6 months) of both the parent drug and its primary metabolite desethylamiodarone, further complicate the clinical use of amiodarone.

Since most of the unfavourable properties of amiodarone are probably due to the presence of its iodine moiety, several noniodinated benzofuran derivatives have been synthesized. Of these modified analogues, dronedarone is most advanced in clinical development [1]. Like amiodarone, dronedarone blocks multiple ion currents and shares the low potential for causing torsades de pointes. Dronedarone has a serum half-life of about 24 h and does not significantly accumulate in plasma or tissue. This also holds true for its principal metabolite debutyldronedarone, which possesses less pharmacologic activity. Recent phase III trials comparing dronedarone treatment with placebo in patients with atrial fibrillation showed efficacy of the drug, while adverse events did not significantly differ between both groups [4,5]. Therefore, dronedarone is expected to receive regulatory approval for several indications concerning atrial fibrillation.

Many assays for the determination of amiodarone and desethylamiodarone in plasma using high-performance liquid chromatography with UV-detection (HPLC–UV) have been described in the literature, of which 40 procedures were compared in a review by Pollak [6]. In recent years, HPLC has also been coupled to mass spectrometry and chemiluminescent detection for even more

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specific and sensitive quantification of amiodarone and desethylamiodarone [7–11]. To the best of our knowledge, no detailed high-performance liquid chromatography (HPLC) assay for the determination of dronedarone and debutyldronedarone has been described so far. Therefore, the primary aim of the present study was to develop an HPLC method for the determination of dronedarone and debutyldronedarone in plasma as well as in the therapeutic target, i.e. in myocardial tissue. Furthermore this assay was validated for determination of amiodarone and desethylamiodarone. Thus we here present a uniform assay that can be applied for detection of both dronedarone and amiodarone as well as their principal metabolites.

2. Experimental

2.1. Chemicals

Dronedarone, debutyldronedarone, amiodarone and desethylamiodarone were a gift from Sanofi-Aventis (Sisteron, France) (Fig. 1). 4,4′-Methylenebis(2,6-diisopropyl-N,N-dimethylaniline) (internal standard) and glycine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Trifluoroacetic acid, ammonia (25%) and sodium hydroxide were obtained from Merck (Darmstadt, Germany). HPLC grade methanol, heptane, dichloromethane, acetonitrile and isopropanol were supplied by Biosolve (Valkenswaard, The Netherlands). A Milli-Q Plus water purification system (Millipore, Amsterdam, The Netherlands) was used to obtain purified water. All other reagents were of analytical grade.

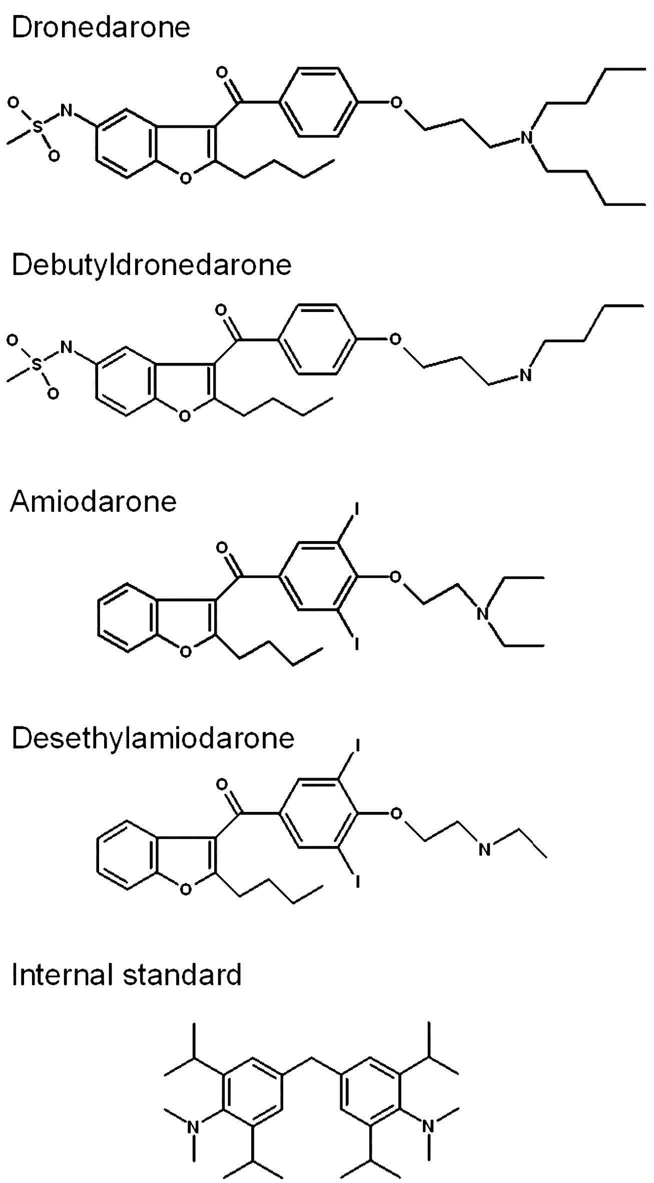

Fig. 1. Chemical structures of dronedarone, debutyldronedarone, amiodarone, desethylamiodarone, and internal standard.

2.2. Biological samples

Samples were obtained from healthy volunteers (plasma) and goats (plasma and left ventricular myocardial tissue). Blood was drawn from a peripheral vein and collected in EDTA-containing vacutainer tubes (Becton-Dickinson, Franklin Lakes, New Jersey, USA) and centrifuged (at 3500 × *g*, 10 min, 4 °C) to obtain plasma. Left ventricular tissue samples were homogenized in water (1:3, w/v) with a tissue blender. Plasma and tissue homogenates were stored at −80 °C.

2.3. HPLC–UV analysis

The HPLC system consisted of a Shimadzu LC-10AD liquid chromatograph, a SIL-10AD autoinjector and a SP8490 variable wavelength UV-detector (Newport Spectra-Physics, Utrecht, The Netherlands) set at 254 nm. LabSolutions software (Shimadzu, Kyoto, Japan) was used for instrument control and data acquisition. HPLC separations were performed on a Pathfinder PS polymeric C18 column (50 mm × 4.6 mm, 2.5 μm) (Shimadzu, Kyoto, Japan) as stationary phase. Each 10-min chromatographic run was carried out at a flow-rate of 1 ml/min with a mobile phase of acetonitrile, isopropanol, water and ammonia (80/10/10/0.025, v/v/v/v).

2.4. Standard solutions

Stock solutions of 2000 μg/ml of dronedarone, debutyldronedarone, amiodarone, desethylamiodarone and internal standard were separately prepared in methanol and diluted and combined to obtain working solutions. Stock solutions were stored at −20 °C and working solutions were kept refrigerated (2–6 °C) and protected from light. The working solutions were used to prepare 12 calibrators in blank plasma (0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.0, 2.0, 3.0, 4.0 and 5.0 μg/ml) and blank tissue homogenates (0.005, 0.01, 0.02, 0.04, 0.08, 0.25, 1.25, 6.25, 12.5, 25, 62.5 and 125 μg/ml, corresponding to wet tissue concentrations of 0.02, 0.04, 0.08, 0.16, 0.32, 1, 5, 25, 50, 100, 250 and 500 μg/g, respectively). Quality control samples were prepared in a similar way with plasma concentrations of 0.04, 1 and 5 μg/ml, and tissue homogenate concentrations of 0.02, 0.25 and 25 μg/ml, corresponding to wet tissue concentrations of 0.08, 1 and 100 μg/g, respectively.

2.5. Extraction procedure

To a 10-ml glass centrifuge tube, 400 μl of plasma or tissue homogenate, 100 μl of working solution (containing 100 μg/ml internal standard), 100 μl of 1 M glycine buffer (pH 1.0) and 5 ml of acetonitrile were added. After precipitation of the protein fraction (at 5000 × *g*, 10 min, 4 °C), the supernatant was transferred to a second 10-ml glass centrifuge tube and evaporated to dryness under nitrogen at 37 °C. To the residue, 1 ml of 1 M glycine buffer (pH 10.5) and 2 ml of a mixture of heptane and dichloromethane (50/50, v/v) were added. After extraction and centrifugation (at 5000 × *g*, 10 min, 4 °C), the supernatant was transferred to a third 10-ml glass centrifuge tube and evaporated to dryness under nitrogen at 37 °C. The residue was reconstituted with 50 μl of mobile