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Multiple modes of inhibition of human cytochrome P450 2J2 by dronedarone, amiodarone and their active metabolites

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ABSTRACT

Dronedarone, a multiple ion channel blocker is prescribed for the treatment of paroxysmal and persistent atrial fibrillation. While dronedarone does not precipitate toxicities like its predecessor amiodarone, its clinical use has been associated with idiosyncratic hepatic and cardiac adverse effects and drug–drug interactions (DDIs). As dronedarone is a potent mechanism-based inactivator of CYP3A4 and CYP3A5, a question arose if it exerts a similar inhibitory effect on CYP2J2, a prominent cardiac CYP450 enzyme. In this study, we demonstrated that CYP2J2 is reversibly inhibited by dronedarone (K_i = 0.034 μM), amiodarone (K_i = 4.8 μM) and their respective pharmacologically active metabolites namely *N*-desbutyldronedarone (NDBD) (K_i = 0.55 μM) and *N*-desethylamiodarone (NDEA) (K_i = 7.4 μM). Moreover, time-, concentration- and NADPH-dependent irreversible inactivation of CYP2J2 was investigated where inactivation kinetic parameters (K_I, k_{inact}) and partition ratio (r) of dronedarone (0.05 μM, 0.034 min^{-1}, 3.3), amiodarone (0.21 μM, 0.015 min^{-1}, 20.7) and NDBD (0.48 μM, 0.024 min^{-1}, 21.7) were observed except for NDEA. The absence of the characteristic Soret peak, lack of recovery of CYP2J2 activity upon dialysis, and biotransformation of dronedarone and NDBD to quinone-oxime reactive metabolites further confirmed the irreversible inactivation of CYP2J2 by dronedarone and NDBD is via the covalent adduction of CYP2J2. Our novel findings illuminate the possible mechanisms of DDIs and cardiac adverse effects due to both reversible inhibition and irreversible inactivation of CYP2J2 by dronedarone, amiodarone and their active metabolites.

1. Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia among geriatric population. As there is a worldwide increase in the incidence and prevalence of AF and its associated mortality and morbidity [1], AF poses a burgeoning socioeconomic burden. For instance, an estimated $6.65 billion USD was spent on AF-related hospitalization such as stroke or thromboembolism in

Abbreviations: AA, arachidonic acid; ACN, acetonitrile; AF, atrial fibrillation; CYP2D6, cytochrome P450 2D6; CYP2J2, cytochrome P450 2J2; CYP3A4, cytochrome P450 3A4; CYP3A5, cytochrome P450 3A5; DDI, drug–drug interaction; EET, epoxyeicosatrienoic acid; GSH, glutathione; k, intrinsic elimination rate constant; K_i, reversible inhibition constant; K_I, inactivator concentration at half-maximum inactivation rate constant; k_{inact}, maximum inactivation rate constant; K_m, Michaelis–Menten constant; K_{obs}, observed rate of inactivation; K_{si}, dissociation constant for the substrate bound to the inhibitory enzymatic site; LC/MS/MS, liquid chromatography in tandem with mass spectrometry; MBI, mechanism-based inactivation; MI complex, metabolite-intermediate complex; MRM, multiple reaction monitoring; NDBD, *N*-desbutyldronedarone; NDEA, *N*-desethylamiodarone; NYHA, New York Heart Association; r, partition ratio; rCYP2J2, recombinant cytochrome P450 2J2; $t_{1/2}$, half-life; TOF/MS, time-of-flight mass spectrometer; USFDA, United States Food and Drug Administration; V_{max}, maximum rate of reaction.

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2005 [2]. AF is characterized by an irregular and rapid heart rate that may occur without any prior cardiac complications (lone AF) or be associated with underlying cardiac diseases such as congestive heart failure, coronary artery disease and hypertension [3]. To date, treatment with pharmacological agents remains clinically relevant with an array of drugs designed to bring about rate or rhythm control. Antiarrhythmic drugs block specific cardiac ion channels and have been classified according to the Vaughan-Williams classification system developed in 1970 [4]. Dronedarone is an antiarrhythmic drug approved by U.S. Food and Drug Administration (USFDA) in 2009 for the treatment of paroxysmal and persistent AF and exhibits pharmacological effects belonging to all four Vaughan-Williams classes [5].

Structurally, dronedarone is similar to amiodarone except for the lack of iodine atoms, the addition of methane sulfonamide group and replacement of tertiary ethylamine group with tertiary butylamine (Fig. 1). It was postulated that as a result of these modifications, dronedarone may not exhibit severe systemic toxicities like interstitial lung disease and thyroid/liver toxicity as commonly observed in amiodarone therapy, while maintaining its multichannel blocking anti-arrhythmic property [6]. However, during Phase III clinical trial, dronedarone was reported to worsen heart failure condition leading to death [7]. As a result, a black box warning has been issued by USFDA advising against the use of dronedarone in AF patients with NYHA Class IV heart failure, Class II/III heart failure patients with recent decompensation or patients with permanent AF [8]. More recently, dronedarone is associated with idiosyncratic proarrhythmia [9], pneumotoxicity [10] and hepatotoxicity [11].

Dronedarone and amiodarone are extensively metabolized by CYP3A4 and CYP3A5 to the pharmacologically active metabolites *N*-desbutyldronedarone (NDBD) and *N*-desethylamiodarone (NDEA) respectively (Fig. 1). Dronedarone has been shown to inhibit CYP3A4 and CYP2D6 moderately [6]. Our laboratory further reported the mechanism-based inactivation (MBI) of CYP3A4 and CYP3A5 by dronedarone and NDBD [12].

Currently, the underlying mechanism of exacerbation of dronedarone-induced cardiac failure is unknown [13]. As extrahepatic CYP450 enzymes are involved in xenobiotic biotransformation and play a dominant role in organ specific pharmacology and toxicology [14], it is imperative to understand the interaction between dronedarone and cardiac specific CYP450 enzymes.

CYP2J2 is a prominent cardiac CYP450 enzyme involved in endobiotic and xenobiotic metabolism [15,16]. As an epoxygenase, CYP2J2 metabolizes arachidonic acid (AA) to bioactive epoxyeicosatrienoic acids (EETs) [17,18] that are associated with numerous cardioprotective functions [18]. Cardiac-specific overexpression of CYP2J2 in transgenic mice was shown to alleviate a number of pathological conditions such as arrhythmic susceptibility in cardiac hypertrophy [19], streptozotocin-induced diabetic cardiomyopathy [20] and doxorubicin-induced cardiotoxicity owing to an increased production of EETs [21]. In other words, CYP2J2 is considered to be a crucial cardiac physiological enzyme.

Previously it was found that amiodarone is both a substrate [16] and an inhibitor of CYP2J2 [22]. However, the mechanism of CYP2J2 inhibition by amiodarone is unknown and a question further arose whether NDEA, dronedarone and NDBD exhibit similar inhibition of CYP2J2.

In this study, we demonstrated both amiodarone and dronedarone are extensively metabolized by CYP2J2, identified specific CYP2J2 metabolites of dronedarone, established reversible and MBI of CYP2J2 by both antiarrhythmic drugs and their metabolites and lastly, identified the putative reactive metabolites involved in dronedarone- and NDBD-mediated inactivation of CYP2J2.

2. Materials and methods

2.1. Chemicals

High-performance liquid chromatography (HPLC)-grade acetonitrile (ACN) was purchased from Tedia Company Inc. (Fairfield, OH). Dronedarone hydrochloride, amiodarone hydrochloride, astemizole, buspirone hydrochloride, danazol, verapamil hydrochloride and glutathione (GSH) were purchased from Sigma–Aldrich (St. Louis, MO). NDBD hydrochloride was purchased from Alsachim Inc. (lllkirch, France), NDEA hydrochloride and ritonavir were purchased from Cayman Chemical (Ann Arbor, MI). Human recombinant CYP2J2 Supersomes™ (rCYP2J2) and NADPH regenerating system consisting of NADPH A (NADP+ and glucose 6-phosphate) and B (glucose-6-phosphate dehydrogenase)

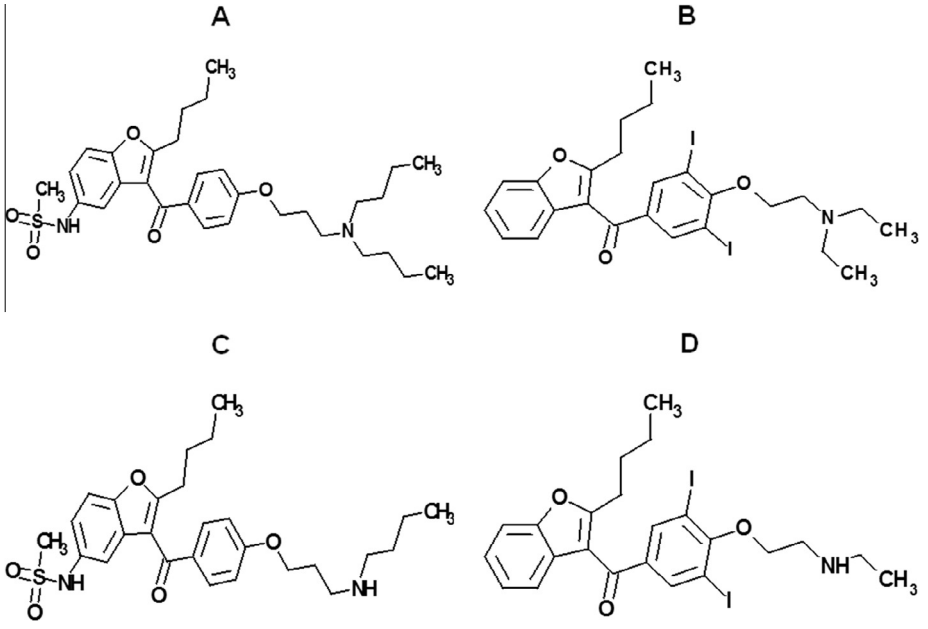

Fig. 1. Chemical structures of (A) dronedarone, (B) amiodarone (C) *N*-desbutyldronedarone and (D) *N*-desethylamiodarone.