Cytochrome P450 2J2: potential role in drug metabolism and cardiotoxicity

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Abbreviations
CYP2J2, cytochrome P450 2J2; EETs, epoxyeicosatrienoic acids; AA, arachidonic acid; eNOS, endothelial nitric oxide synthase; DOX, doxorubicin; ROS, reactive oxygen species; sEH, soluble epoxide hydrolase; DHETs, dihydroxyeicosatrienoic acids; SNPs, single nucleotide polymorphisms; VCAM-1, vascular cell adhesion molecule-1; CRH, coronary reactive hyperemia; mPTP, mitochondrial membrane permeability pore; LVDP, left ventricular developed pressure; BNP, beta natriuretic peptide; ANP, atrial natriuretic peptide; NDBD, N-desbutyldronedarone (NDBD); TdP, torsades de pointes; CARP, cardiac ankyrin repeat protein;
Abstract

Drug-induced cardiotoxicity may be modulated by endogenous arachidonic acid (AA) derived metabolites known as epoxyeicosatrienoic acids (EETs) synthesised by cytochrome P450 2J2 (CYP2J2). The biological effects of EETs including their protective effects on inflammation and vasodilation are diverse due, in part, to their ability to act on a variety of cell types. In addition, CYP2J2 metabolises both exogenous and endogenous substrates and is involved in phase I metabolism of a variety of structurally diverse compounds including some antihistamines, anti-cancer agents and immunosuppressants. This review addresses the current understanding of the role of CYP2J2 in metabolism of xenobiotics and endogenous AA with particular focus on the effects on the cardiovascular system. In particular, the hypothesis that CYP2J2 influences drug-induced cardiotoxicity through potentially conflicting effects on the production of protective EETs and metabolism of drugs is promoted here.
Introduction

Drug-induced cardiotoxicity affects all components and functions of the cardiovascular system. It is characterized by changes in ECG waveform morphology, haemodynamics, pathological damage to the myocardium and vasculature and changes in blood function (Laverty et al., 2011). Cardiotoxicity is a major cause of attrition in preclinical and clinical drug development, and may be attributed to a number of mechanisms (Pointon et al., 2013). Drug exerting effects on the cardiovascular system have been shown to change heart contractility, cardiac rhythm, blood pressure and ischaemia (Feenstra et al., 1999). Anti-histamines such as astemizole and terfenadine cause abnormalities in ECG wave intervals such as an increase in QT interval leading to Torsades de Pointes (Zhou et al., 1999, Lu et al., 2012). The anti-cancer drug doxorubicin however causes oxidative stress resulting in structural damage to the heart leading to heart failure (Chatterjee et al., 2010). Whereas astemizole primarily exerts its effect on cardiomyocytes, doxorubicin (DOX) also affects ancillary cells such as fibroblasts, endothelial cells and vascular smooth muscle cells, disrupting the structure and function of vascular smooth muscle cells and inducing a pro-fibrotic phenotype in fibroblasts (Chatterjee et al., 2010).

This review focuses on cytochrome P450 2J2 (CYP2J2) which has been shown to modulate drug-induced cardiotoxicity (Zhang et al., 2009b). The biological role of CYP2J2 appears to relate primarily to its metabolism of arachidonic acid (AA) to cardio-protective epoxyeicosatrienoic acids (EETs). Evidence is presented within this review to support the proposal that a complex interplay between EET synthesis and drug metabolism by CYP2J2 exists. It is likely that EET synthesis often predominates and largely protects the cardiovascular system but that drugs might inhibit EET synthesis in a competitive or non-competitive manner; or else drug metabolism by CYP2J2, in the heart or elsewhere, might liberate cardiotoxic drug metabolites.
EETs possess pleiotropic biological activities including stimulation of angiogenesis, vasodilation, inhibition of vascular smooth muscle cell migration, protection against hypoxia-reperfusion injury, increased endothelial nitric oxide synthase (eNOS) expression and activity, and protection against doxorubicin (DOX)-induced cardiotoxicity (Larsen et al., 2007, Spector and Norris, 2007, Yang et al., 2009, Zhang et al., 2009b, Campbell and Fleming, 2010). Given these wide-ranging effects on the cardiovascular system it is not surprising that EETs and CYP2J2 might modulate the pathogenesis of cardiovascular disease. However, the understanding behind the protective role of EETs during cardiotoxicity is relatively unexplored suggesting that further studies on a range of cardiotoxic agents are worthwhile. CYP2J2 is also a drug metabolising enzyme and has been implicated in the biotransformation of a variety of drugs in the liver and other tissues (Xu et al., 2013, Michaud et al., 2010). We also highlight how the balance between drug metabolism and protective EET formation may influence cardiotoxicity (Figure 1).

**Role of CYP2J2 in cardiovascular biology**

The mRNA expression of CYP2J2 in humans is mainly confined to the cardiovascular system and liver, with predominant expression in the right ventricle of the heart (Michaud et al., 2010). However mRNA has also been demonstrated in the kidney (Enayetallah et al., 2004), mRNA and protein in the lung (Zeldin et al., 1996), brain (Dutheil et al., 2009), GI tract (Zeldin et al., 1997b), pancreas (Zeldin et al., 1997a) and some human carcinoma tissues at lower levels (Jiang et al., 2005). Discrepancies between CYP2J2 mRNA and protein expression have been found in the liver (Gaedigk et al., 2006), the consequence for the heart remains unknown. Additionally, multiple immunoreactive bands on Western blotting from extracts of adult human liver and heart have been reported. It has been hypothesised that these are uncharacterised isoforms of CYP2J2 (Wu et al., 1996, Gaedigk et al., 2006). These isoforms may possess similar or alternate activities to the main isoform of CYP2J2 and so warrant further study. Despite its elevated expression in the cardiovascular system compared to
other tissues, the role of CYP2J2 in the metabolism of drugs in the heart is, to an extent, still unknown.

Results from a cytochrome P450 mRNA screen showed that CYP2J2 is the predominant isoenzyme expressed in cardiomyocytes and human heart tissue (Figure 2) (Evangelista et al., 2013). Furthermore, in line with these high levels of mRNA expression, CYP2J2 protein levels in human heart microsomes were approximately 50 fold higher than other P450 enzymes (Evangelista et al., 2013, Bylund et al., 2001). Evangelista also established mRNA levels of the P450 enzyme CYP4F12 in human heart. Cytochrome P450 enzymes belonging to the CYP4A and 4F subfamily produce 20-hydroxyeicosatetraenoic acid (20-HETE) from AA (Miyata et al., 2005, Harmon et al., 2006, Tang et al., 2010) and show increased expression in cardiovascular disease. 20-HETE has antagonistic effects towards EETs, exacerbating disease processes (Jenkins et al., 2009). However, it is largely unknown how these other enzymes contribute to cardiovascular function and whether they have a role in cardiotoxicity.

In addition to cardiomyocytes, CYP2J2 expression has also been observed in other cardiovascular cells. CYP2J2 is expressed in endothelial cells in a variety of vascular beds including coronary artery, aorta (Delozier et al., 2007) and in varicose veins (Bertrand-Thiebault et al., 2004). The presence of CYP2J2 in a variety of tissues and specific expression in the heart leads to the hypothesis that CYP2J2 could contribute to endogenous tissue function. Although it is not well explored, the expression of CYP2J2 in endothelial cells, fibroblasts and smooth muscle cells (Deb and Ubil, 2014, Brutsaert, 2003) could contribute to cardiotoxicity through drug metabolism as well as protective effects through the formation of EETs.

In endothelial cells, mRNA for another epoxygenase, CYP2C9 is highly expressed compared to CYP2J2 (Delozier et al., 2007). Moreover, CYP2C9 mRNA was higher both in human aorta and coronary
artery than CYP2J2 and CYP2C8. Protein analysis further reflected this, as CYP2C9 expression was dominant compared to CYP2J2 and expression of CYP2C8 was not observed (Delozier et al., 2007). Although CYP2C9 can produce EETs, it has also been reported to generate damaging reactive oxygen species (ROS). ROS are regarded as pro-inflammatory mediator, increasing NF-κB activity (Fleming, 2001) presumably leading to increased expression of pro-inflammatory cytokines, growth factors and adhesion molecules, inducing an inflammatory phenotype and opposing the effects of CYP2J2 (Taniyama and Griendling, 2003).

CYP2J2 catalyses the epoxidation of the double bonds of endogenous cellular AA to generate EETs (Figure 3) (Zeldin et al., 1997b). Due to the four double bonds of AA and the stereochemistry and regiochemistry associated with these bonds, 8 EET isomers are possible: 5,6-EET (R/S), 8,9-EET (R/S), -11,12-EET (R/S), 14,15-EET (R/S) (Xu et al., 2013). In vivo EETs are rapidly metabolised by soluble epoxide hydrolase (sEH) to dihydroxyeicosatrienoic acids (DHETs) which are less biologically active (Zhang et al., 2014). In addition to DHET formation, re-esterification of EETs and incorporation into the phospholipid membrane for storage occurs allowing release and distribution (Bernstrom et al., 1992). Other relatively minor metabolism pathways of EETs have been described involving cyclooxygenase, lipoxygenase and CYP ω-oxidase activities (Spector et al., 2004).

Soluble epoxide hydrolase may have significant biological activity in a variety of tissues including the liver, kidney, heart, spleen, endothelium and mammary gland (Newman et al., 2005). Its deactivation of biologically protective EETs has led to the development of pharmacological inhibitors of sEH (Liu et al., 2009, Hwang et al., 2013). This resulted in the first sEH inhibitor, AR9281, to begin phase Ila trials for the treatment of type 2 diabetes (Anandan et al., 2011) as well as recent introduction of sEH inhibitor, GSK225629, which is currently in phase I trials for the treatment of hypertension (Lazaar et al., 2016) and COPD (Yang et al., 2017). Furthermore, several studies have shown that sEH
plays an influential role in the development of myocardial hypertrophy (Xu et al., 2006) and atherosclerosis (Zhang et al., 2009a).

Investigation of the CYP2J2 epoxidation pathway in various tissues has provided evidence to support the theory that CYP2J2, and thus EETs, have a biologically protective role and this is emphasised by the growing potential of sEH inhibitors. The investigation of these small molecule inhibitors suggests a link between sEH inhibition and improved cardiovascular health and given the high expression of CYP2J2 in the human heart, suggest a possible protective role for CYP2J2 in drug-induced cardiotoxicity.

**Genetic variation in human CYP2J2**

Epidemiologic studies conducted to examine variants in many cytochrome P450 genes have found over 2000 single nucleotide polymorphisms (SNPs) (Preissner et al., 2013). A number of these have been associated with disease. Within the Chinese population, a proximal promoter polymorphism (-50G>T), rs890293 (CYP2J2*7), has been shown to alter CYP2J2 expression (Table 3). This mutation has been shown to decrease binding of Sp1 transcription factor to the promoter region of CYP2J2. As Sp1 is responsible for regulating transcriptional basal activity, blocking it results in a ~50% reduction in promoter activity and decreased expression of the CYP2J2 gene (Spiecker et al., 2004). This polymorphism may be involved in the pathogenesis of type 2 diabetes (Wang et al., 2010), Alzheimer’s disease (Yan et al., 2015), chronic kidney disease and was shown to be negatively associated with cardiovascular diseases including myocardial infarction (Jie et al., 2010), coronary artery disease (Zhu et al., 2013) and hypertension (Wu et al., 2007) within this population. Contrary to this, studies conducted in the Swedish and German populations looking at cardiovascular risk found no susceptibility to hypertension, coronary artery disease or stroke in carriers of the rs890293 polymorphism, (Fava et al., 2010, Hoffmann et al., 2007) indicating more association studies may be
required to elucidate the risk of this CYP2J2 polymorphism for cardiovascular disease. None of the other SNPs in CYP2J2 has been shown to be associated with disease (Table 3). Furthermore, there have been no reported associations between any CYP2J2 polymorphisms and cardiotoxicity despite some of the variants having profound effects on enzyme expression or activity in vitro. Without further study it is unknown whether CYP2J2 polymorphisms may be important in cardiotoxicity.

CYP2J2/EETs in the maintenance of cardiovascular health- a potential role in cardiotoxicity?

Cardiotoxicity can be viewed as a continuum of physiological states that shares characteristics with cardiovascular disease. Consequently, understanding the role of CYP2J2 and EETs in cardiovascular disease can provide insight into their role in cardiotoxicity. It is widely recognised that many cytochrome P450 enzymes, are upregulated in failing hearts (El-Kadi and Zordoky, 2008). The up-regulation of CYP2J2 and EETs has been shown to be protective in the heart. In the following sections we discuss how CYP2J2 may play a role in modulation of vascular inflammation, vascular tone, ischaemia reperfusion injury and cardiac hypertrophy.

Vascular Inflammation

Infiltration of inflammatory cells, particularly monocytes/macrophages has been shown to be an early event in and be causal in the development of cardiovascular pathologies. Potent anti-inflammatory effects of CYP2J2 and EETs have been demonstrated both in vivo and in vitro. In vitro, synthetic EETs can reduce expression of many pro-inflammatory genes which are involved in activation and adhesion of endothelial cells to leukocytes and leukocyte transmigration across the endothelium (Xu et al., 2011). For example, 11,12-EET suppresses expression of adhesion molecules, E-selectin and vascular cell adhesion molecule-1 (VCAM-1), in TNF-α induced human endothelial cells (Node et al., 1999). In addition to blocking the actions of TNF-α, EETs (11, 12- and 8, 9-EET) have also
been shown to inhibit basal TNF-α production in THP-1 cells, a model monocytic cell line (Bystrom et al., 2011). EETs inhibit LPS-induced macrophage polarisation and reduce expression of many proinflammatory cytokines whilst at the same time upregulating anti-inflammatory cytokine IL-10 in HEK293 cells over-expressing recombinant CYP2J2; these effects are likely mediated through downregulating NF-κβ and activation of PPAR receptors (Dai et al., 2015).

Studies in mouse models have further validated the effects of transgenic CYP2J2 and EETs in inflammation. Continuous infusion of 11,12-EET inhibited TNF-α-induced endothelial VCAM-1 expression and mononuclear cell rolling and adhesion in mouse coronary arteries. In addition, in a CYP2J2 transgenic mouse model expression of CYP2J2 in mice significantly reduced LPS-induced production of pro-inflammatory mediators, IL-6, MCP-1, E-selectin, and IL-1β, as well as NF-κB activation and invasion of inflammatory cells in lung tissues (Potente et al., 2003). EETs inhibited phosphorylation of the NF-κB complex preventing its translocation to the nucleus and hence transcriptional effects (Node et al., 1999). Transgenic CYP2J2 was also found to reduce Ang II-induced cardiac fibrosis and inflammation in mice possibly though the inhibition of the NF-κB pathway (Yang et al., 2015).

Inflammatory processes have been implicated in the development of atherosclerosis. CYP2J2 has also been found to protect against the production of atherosclerotic plaques in a transgenic mouse model of atherosclerosis (Liu et al., 2016). Apo-E deficient mice and CYP2J2 transgenic mice were given a high fat diet for 25 weeks. Histological analysis of aorta samples identified fewer plaques on the luminal surface of the aorta when mice had been injected with CYP2J2 vector compared to wild type. Analysis of lipids showed lower circulating triglyceride and cholesterol levels in the transgenic mice. 11,-12 EET was also shown to inhibit TNF-α induced apoptosis in HUVECs through the activation of AKT and FOXO1 which is down regulated in atherosclerotic aorta (Liu et al., 2016). However it is challenging to ascertain whether these results are a direct outcome of CYP2J2 derived
EETs or other protective molecules, for example, metabolites of EPA and DHA; moreover, to ascertain which of the EETs may be responsible for the protective action.

Likewise, one of the hallmarks of some drug-induced cardiotoxocities is the increase in production of inflammatory mediators. For example epirubicin, known to alter cardiac morphology, increases IL-6 and its soluble receptor sIL-6R which have been shown to contribute to the pathophysiology of cardiomyopathy (Mercuro et al., 2007). It is possible that the anti-inflammatory effects of EETs could attenuate some of the myocardial damage mediated by elevated levels of these inflammatory mediators.

Vascular tone

Maintenance of vascular tone is critical for cardiovascular function, particularly contributing to blood pressure regulation. EETs, in particular 11,12-EET, are also known as endothelial derived hyperpolarising factors (EDHFs) and have been shown to cause relaxation of rat coronary arteries as well as renal and cerebral arteries of rats and rabbits (Larsen et al., 2006, Imig et al., 2001, Fisslthaler et al., 1999, Dimitropoulou et al., 2007, Campbell et al., 1996). EETs have been shown to reduce vascular tone by attenuating calcium entry via voltage-sensitive channels leading to hyperpolarization of vascular smooth muscle cells. However, EETs may also increase intracellular calcium concentration in endothelial cells by activation of KCa channels.

Although an increase in EETs has been shown to reduce vascular tone and protect against cardiovascular disease in animal models, it is still unknown how this protective pathway may influence cardiotoxicity

Ischaemia reperfusion injury

Ischaemia–reperfusion injury can lead to accumulation of protective EETs following the release of fatty acids by membrane bound phospholipases (Seubert et al., 2004). Increasing EETs in a sEH null
mouse model showed a limited mitochondrial damage following ischaemia compared to wild type (Akhnokh et al., 2016). EETs can enforce their cardioprotective effects through the activation of mitoK (ATP) and opening of the mitochondrial membrane permeability pore (mPTP) (Barau et al., 2015). Activation of this protective pathway by EETs has been shown to maintain mitochondrial structure and function in CYP2J2 transgenic mice (Seubert et al., 2004). It is well known that mitochondrial ischaemia reperfusion injury activated apoptosis. EETs have been shown to inhibit pro-apoptotic pathways through the pro-survival enzyme phosphoinositide 3 kinase (PI3K) in rat cardiomyocytes (Isomoto et al., 2006).

Transgenic mice with endothelial cell specific CYP2J2 expression identified that endothelial-derived EETs did not protect against cardiac ischaemia; moreover, transgenic mice with enhanced endothelial expression of sEH showed no changes in left ventricular developed pressure (LVDP) and infarct size. However, transgenic mice with myocardial specific expression of CYP2J2 had increased recovery of LVDP and decreased infarct size after ischaemia-reperfusion compared to wild type. Furthermore, transgenic mice with expression of myocardial sEH showed no changes in LVDP and infarct size (Edin et al., 2011). This provides a further layer of complexity to the protective capabilities of EETs and how EETs produced from different areas and cell types of the cardiovascular system may have varying effects.

**Cardiac Hypertrophy**

Cardiac hypertrophy is a prominent risk factor for heart failure and a strong predictor of adverse cardiovascular events (El-Kadi and Zordoky, 2008). It is normally characterised by an increase in cardiomyocyte size, increased synthesis of beta natriuretic peptide (BNP), atrial natriuretic peptide (ANP), myosin and actin accompanied by fibrosis and remodelling (Alsaad et al., 2013). Animal models have shown that during isoproterenol-induced cardiac hypertrophy there is a decrease in protective EETs. Modulation of this process, that is, increasing EET half-life by use of sEH inhibitors,
protected against the detrimental effects of cardiac hypertrophy although the exact mechanism for this is yet to be determined. (El-Kadi and Zordoky, 2008).

**Animal homologues of CYP2J2 to investigate pre-clinical drug-induced cardiotoxicity**

Prior to first time in human, novel small molecules have to be tested in both rodent and non-rodent in vivo models to assess for potential safety liabilities in major organs including the heart. Many mammals possess homologs of the human CYP2J2 protein with varying sequence similarities including the commonly used species for regulatory safety pharmacology and toxicology studies (rodent, dog and monkey) (Table 1 and Table 2).

Compared to the single CYP2J2 gene in humans, mice have a cluster of CYP2J isoform genes. This subfamily is highly homologous with 62-84% homology at the amino acid level compared to human. Mice CYP2J isoforms are distributed in the liver, kidneys, intestine, brain, lung and abundantly in the heart (Graves et al., 2013). All enzymes produced from the CYP2J cluster have similar substrate preferences but the products produced have a unique profile (Nelson et al., 2004). Compared with recombinant CYP2J2 microsomes, all isoforms have been shown to metabolise AA albeit at a lower rate (Graves et al., 2013).

Mouse models are used to investigate cytochrome P450-dependent metabolism. Knockout and transgenic mice are used to study the metabolism pathways pertaining to a specific enzyme leading to toxicity. For example, knockout and humanised mouse models for CYP2E1 have been used to characterise acetaminophen hepatotoxicity (Gonzalez et al., 2015). Currently, transgenic mouse models for CYP2J have been created to understand the biological significance of EETs in disease. However, studies specifically addressing the role of CYP2J in the induction of toxicity have not yet
been described. In vitro, mouse derived HL-1 cell lines have limited cardiac morphological, biochemical and electrophysiological properties compared to human adult cardiomyocytes. However, their expression of P450 enzymes has not been clearly investigated and so may offer a potential in vitro cardiac model to study CYP2J.

The main rat homologues, CYP2J3 and CYP2J4, have 72% and 76% sequence similarity to human CYP2J2 and have a similar tissue distribution to human. Furthermore, CYP2J3 is reported to be found primarily within atrial and ventricular myocytes. (Wu et al., 1997, Zhang et al., 1997), while increased expression of CYP2J3 in the heart following ischemic postconditioning significantly increased EET generation (Wang et al., 2012) suggesting CYP2J3 may have epoxygenase activity analogous to CYP2J2. Therefore, rat CYP2J3 may be the closest homologous enzyme to CYP2J2 in terms of distribution and epoxygenase activity and may be applicable to investigations of cardiotoxicity. Also, rat cardiac cell lines such as rat myoblast H9c2 cells have been used to investigate cardiac biology and toxicology, however they lack key functional features of cardiomyocytes, exhibit a mainly skeletal muscle phenotype and do not respond to electrical stimulation (Kimes and Brandt, 1976). However, they have recently been used to investigate cardioprotective effects of drugs following oxidative damage (Zhou et al., 2016), thus suggesting the potential use of these cells in investigating mechanisms of cardioprotection from drug induced cardiotoxicity.

In both dog and monkey, a single CYP2J isoform (CYP2J2) has been identified (Nelson, 2009). Monkey CYP2J2 has the greatest sequence similarity (95%) to CYP2J2 in humans (Uno et al., 2007). Immunoquantification of cynomolgus CYP2J2 identified higher levels of protein in monkey liver compared with human (Uehara et al., 2015). This was reflected in activity where there was a higher hepatic clearance of astemizole (Nishimuta et al., 2011) which has been shown to be a drug
substrate for CYP2J2 (Uehara et al., 2015). However, to our knowledge, expression of CYP2J2 at the mRNA or protein level has not yet been quantified in the monkey or dog heart.

Cardiotoxicity encompasses a variety of features including changes in pathology, ECG and haemodynamics. Therefore, some animal models may be recommended for investigating functional changes whereas other models may be more suited to look at pathological changes. For example, rats which reflect the protective capabilities of CYP2J would be acceptable for studying EETs, however their use in predicting cardiotoxicity associated with ion channel inhibition is limited. Consequently, when selecting a suitable preclinical model system both the potential ability to modulate CYP2J2 and the expected cardiac effects being risk assessed should be taken into consideration in selecting the most appropriate approach and species. Furthermore, although dog has a single CYP2J2 isoform with high sequence similarity more studies are required to fully determine if dog is the best model to study CYP2J2, from both a cardiovascular biology and CYP2J2 perspective.

**Role of CYP2J2 in xenobiotic metabolism**

In the human liver, CYP2J2 protein comprises of 1 to 2% of total P450 content similar to that in the small intestine (1.4%) (Paine et al., 2006) compared with CYP3A4 that makes up ~30% of total P450 content (Michaels and Wang, 2014). However as CYP3A4 expression is low in cardiac tissue it is unlikely to contribute to drug metabolism within the heart (Chaudhary et al., 2009). Although not the most highly expressed cytochrome P450 in the liver and intestine CYP2J2 has been shown to mediate drug biotransformation reactions with a number of exogenous substances. CYP2J2 has been shown to be the primary enzyme involved in several metabolic reactions including amiodarone 4-hydroxylation, astemizole O-demethylation and ebastine hydroxylation (Matsumoto et al., 2002, Matsumoto and Yamazoe, 2001, Liu et al., 2006). For ebastine, CYP2J2 plays a superior role in first-pass intestinal metabolism to its pharmacologically active metabolite and less toxic carebastine. All
three of these compounds are known cardiotoxins. Both astemizole and ebastine block the Kv11.1 (hERG) potassium channel, causing torsades de pointes and QT prolongation arrhythmias. However both amiodarone and astemizole metabolites are as toxic as the primary compound when metabolised (Matsumoto et al., 2002). Thus the individual product profile of a compound due to CYP2J2 metabolism may lead to less toxic or equipotent metabolites and altered toxicity of these drugs within the heart.

A study in human liver microsomes identified 8 novel substrates for CYP2J2 after screening 139 compounds including marketed therapeutic agents. These chemical entities had wide structural diversity and ranged from small molecules like albendazole to larger complex structures such as cyclosporine (Lee et al., 2010). This diversity in drug substrates highlights how CYP2J2 may be vital in mediating drug responses and gives a glimpse into the similarity between CYP2J2 and other P450 enzymes of similar function. CYP2J2 and CYP3A4 share a number of substrates including anti-histamines (terfenadine, astemizole and ebastine), anticancer drugs (doxorubicin and tamoxifen) and immunosuppressants (cyclosporine); a list of known CYP2J2 substrates is shown in Table 4. In silico approaches suggest structural similarity between CYP2J2 and CYP3A4 and a comparison of active sites showed homology; however further examination shows slight differences in structural geometry. CYP2J2 has a more cylindrical shape and is narrower than CYP3A4 as the β-4 part of the protein is smaller and has a loop which inserts into the active site restricting metabolism. (Lee et al., 2010).

Biotransformation studies looking at the metabolism profile of CYP2J2 and CYP3A4 showed that CYP2J2 produced numerous metabolites, many of which were also observed with CYP3A4 (Figure 4). However, indications of differences in the regioselectivity in metabolites from albendazole, amiodarone thioridazine, mesoridazine, danazol and astemizole, after incubation with the two enzymes were found. There was also evidence some metabolites were produced exclusively by
CYP2J2. (Lee and Murray, 2010). Further investigation by Kaspera et al. (2014) showed the significant contribution of CYP2J2 to ritonavir metabolism in the liver, with a unique metabolism profile when compared with CYP3A4/5. CYP2J2 was shown to produce specific metabolites from the oxidation of the thiazole rings on different sides of the molecule. This study found that CYP2J2 had a higher affinity for ritonavir (Km 0.016µM) compared to CYP3A4 (Km 0.068µM) and CYP3A5 (Km 0.047µM) in liver microsomes (Kaspera et al., 2014). In addition, CYP2J2 and CYP2C19 were found to be the major enzymes responsible for the metabolism of albendazole and fenbendazole in human liver microsomes. Both of these drugs can be transformed to their sulfoxide and hydroxyl metabolites (Wu et al., 2013). CYP3A4 and flavin-containing monooxygenase are thought to be major enzymes in producing sulfoxide metabolites (Virkel et al., 2004). However, Zhexue et al., (2013) demonstrated that CYP2J2 was the primary enzyme mediating albendazole hydroxylation; CYP2C19 and CYP2E1 also contributed to this hydroxylation but to a lesser extent. (Wu et al., 2013). The consequences of the formation of these specific metabolites by CYP2J2 has yet to be fully determined.

Several CYP2J2 substrates are known to have pharmacological effects in the heart and may be metabolised in this tissue (Evangelista et al., 2013). Applying this logic, CYP2J2 may be able to regulate the local concentrations of these compounds and therefore modulate cardiotoxicity. Studies in heart microsomes incubated with verapamil led to the formation of nine CYP metabolites. As verapamil is an L-type calcium channel blocker, which is commonly prescribed for heart conditions such as angina and arrhythmias, CYP2J2 may be able to regulate functional activity of the drug (Michaud et al., 2010). Furthermore, in isolated rat heart hydroxylation of the H1 receptor antagonist, ebastine to hydroxyebastine and carebastine was detected which when compared to human liver microsomes showed a similar metabolism profile. However, as there was no comparison to metabolism in the human heart it is difficult to ascertain comparative activity between CYP2J2 and CYP2J3. It is also unclear whether other P450 enzymes could be responsible, in part, for ebastine metabolism (Kang et al., 2011). Overall, the metabolic activity of CYP2J2 in the liver and its ability to
metabolise a wide array of drugs, coupled with its high expression in the heart, warrant further studies to clarify the significance of cardiac CYP2J2 in drug metabolism in physiological relevant systems.

**Modulation of CYP2J2 activity by drugs**

Evangelista et al. (2013) investigated the role of different drugs in the induction and inhibition of CYP2J2 in adult primary human cardiomyocytes. That the cells used in this study are able to divide and are morphologically and functionally different compared with freshly isolated cells is a major limitation of this research. CYP2J2 activity was measured via terfenadine hydroxylation at two different inhibitor concentrations. The most potent inhibitor of CYP2J2 tested was danazol which reduced activity by ~95%; other less potent inhibitors included ketoconazole and astemizole (Evangelista et al., 2013). In addition to the inhibitors of CYP2J2 recognised in this review other drugs are reviewed elsewhere (VanAlstine and Hough, 2011, Lafite et al., 2007).

A more recent study highlighted the reversible mixed-mode inhibition of recombinant CYP2J2 by dronedarone (Ki =0.034µM), amiodarone (Ki =4.8µM) and their active metabolites, N-desbutyldronedarone (NDBD) (Ki =0.55µM) and N-desethylamiodarone (NDEA) (Ki=7.4µM) and further irreversible inhibition by dronedarone and NDBD (Karkhanis et al., 2016). Both of these drugs are multi-ion channel blockers designed to reduce cardiac arrhythmias but paradoxically are potentially also cardiotoxic resulting in bradycardia, hypotension, congestive heart failure and ventricular tachycardia (Dixon et al., 2013). Dronedarone has been demonstrated to cause a reduction in a recurrent atrial fibrillation in patients compared to amiodarone (Piccini et al., 2009) but can lead to QT prolongation and subsequently torsades de pointes (TdP) in some instances (Heijman et al., 2013). As both amiodarone and dronedarone are substrates for CYP2J2, the interaction between these drugs and CYP2J2 may modulate cardiac side effects and drug-drug
interactions leading to further toxicity. Dronedarone, amiodarone and their respective metabolites have been shown to inhibit CYP2J2 mediated arachidonic acid metabolism and production of EETs NDBD being the most potent (Karkhanis et al., 2017). Due to the protective role of CYP2J2 in cardiac function it is feasible that the inhibition of CYP2J2 may block epoxygenase activity and reduce protective EETs, although our understanding of the interplay between drug metabolism and EET formation is rudimentary. In addition, it is possible that modulation of CYP2J2 activity may lead to altered levels of toxic parent compound or toxic metabolite and changes in the toxicity profile observed. These concepts are worthy of further study and analysis.

Potential role of EETs in mitigating drug induced cardiotoxicity

The mechanisms by which compounds cause cardiotoxicity resulting in changes in ECG waveforms / intervals, hemodynamics or cardiac pathology are diverse and compound specific. Each of these outcomes can be further defined both in terms of the physiological and pathological state and the molecular mechanism and or pathways by which perturbations arise. Currently the level of molecular understanding varies considerably depending on the physiological or pathological perturbation. For example, the ion channels behind the cardiac action potential and thus changes in the ECG are well defined. In contrast, multiple mechanisms are proposed for changes in cardiac pathology. For example, the anti-cancer tyrosine kinase inhibitor sunitinib causes oxidative stress (Aparicio-Gallego et al., 2011) and cardiac hypertrophy (Maayah et al., 2014); whether these perturbations are linked and if upstream unidentified mechanisms exist still remains to be determined. However, with this diversity of molecular mechanisms responsible for cardiotoxicity, the role of EETs in cardio-protection is just as diverse (table 5). In an animal model of isoproterenol-induced cardiac hypertrophy, use of sEH inhibitors increased levels of EETs and decreased the induction of ANP and BNP and EPHX2 mRNA (Althurwi et al., 2013). Furthermore, increased circulating EETs reduced oxidative stress and increased expression of antioxidant enzymes (Wang et
Therefore there is some evidence to suggest protection against sunitinib (and other drug) toxicity may be achieved by an upregulation of these epoxygenases with resultant promotion of EET formation.

Other cardiotoxic drugs such as the anti-arrhythmic drug amiodarone have been shown to alter apoptotic pathways, increasing caspase 3 activity leading to an increase in apoptosis and cell death in h9c2 cells (Isomoto et al., 2006). The addition of exogenous EETs in vitro has been shown to boost activity of the pro-survival enzyme phosphoinositide 3 kinase (PI3K) and inhibit pro-apoptotic pathways in primary cardiomyocytes derived from a rat heart hypoxia/reperfusion model (Dhanasekaran et al., 2008).

Cardiotoxic drugs also impact haemodynamics within the cardiovascular system. An example of detrimental effects of drugs on blood pressure is the CYP2J2 substrate 5-fluorouracil which has been shown to be vasoconstrictive through inhibition of nitric oxide synthase leading to coronary spasms, and via protein kinase C leading to vasoconstriction (Alter et al., 2006). Inhibition of sEH increased EETs and dilated human coronary arterioles through BK<sub>ca</sub> channels and 11,12-EET also caused relaxation of rat coronary arteries and renal and cerebral arteries of rats and rabbits. (Larsen et al., 2006, Imig et al., 2001, Fisslthaler et al., 1999, Dimitropoulou et al., 2007, Campbell et al., 1996).

Taken together, EETs have been shown to act on several pathways involved in cardiotoxicity and there is circumstantial yet plausible evidence to suggest a protective impact of CYP2J2 via EET synthesis in the heart during cardiotoxicity.

**Role of CYP2J2 in DOX induced cardiotoxicity**

The strongest case for a role of CYP2J2 in protection against cardiotoxicity has been presented for DOX. DOX is an anthracycline used for the treatment of solid tumours and haematological carcinomas which has been shown to modulate CYP2J2 production of EETs. Despite its anticancer
action, the clinical value of this drug is reduced due to acute and chronic, cumulative and irreversible
dose dependent cardiotoxicity (Belham et al., 2007). Cardiovascular effects include acute
cardiomyopathy, chronic heart failure, ventricular dysfunction and arrhythmias (Yeh et al., 2004).
Currently it is unclear whether the mechanism of cardiotoxicity is the same, when occurring acutely
(within days) or chronically (years) following treatment (Takemura and Fujiwara, 2007).

Preclinically, both acute and chronic DOX administration have been associated with changes in EET
formation (Zordoky et al., 2010, Alsaad et al., 2012). In rats, acute DOX treatment was found to alter
the mRNA expression of P450 and sEH enzymes in kidney and liver, leading to decreases in 5,6-, 8,9-, 11,12-
and 14,15-EET (Zordoky et al., 2010). Ichikawa et al., (2014) found that acute DOX induced
cardiotoxicity was associated with generation of reactive oxygen species, cellular iron accumulation
and disruption of the mitochondria which in turn initiated apoptotic pathways in isolated neonatal
rat cardiomyocytes (Ichikawa et al., 2014). Previously, EETs have been shown to attenuate reactive
oxygen species production and mitochondrial dysfunction in carcinoma cells treated with arsenic
trioxide (Liu et al., 2011) and an interesting and potentially important recent study suggested that
CYP2J2 mRNA expression increased in adult human ventricular myocytes in culture in response to
both external oxidants and addition of doxorubicin; the latter, amongst other effects, stimulates
intracellular oxidant production. Moreover, cell survival decreased with oxidant exposure when
CYP2J2 was inhibited either using danazol or by siRNA for CYP2J2. Unsurprisingly, this reactive
oxygen species toxicity was mitigated with the addition of exogenous EETs. Although the report is
intriguing and plausible, there are limitations to be highlighted. The use of millimolar concentrations
of pyruvate as the only antioxidant trialled limits mechanistic interpretation as does the use of
danazol as a CYP2J2 inhibitor; however in support of CYP2J2-derived EETs as protective agents,
knockdown of CYP2J2 mRNA also negatively affected cell survival following DOX yet levels of CYP2J2
protein were not addressed. The relatively non-specific measurement of intracellular oxidant
formation resulting from DOX treatment of cells also limits interpretation of the mechanism of
molecular signalling. Nevertheless, this mechanism may have implications for other cardiotoxic compounds acting, in part, via oxidant formation in cells of the cardiovascular system. If the mechanism is proven to be mediated by increased oxidants in cells then protection from these agents could also be afforded by changes in CYP2J2 gene expression and the resultant increased EET formation.

Alsaad et al. (2012) studied the effects of chronic DOX cardiotoxicity in the heart on the mRNA expression of proteins involved in the formation of AA metabolites as well as levels of these metabolites via LC/MS in rats. Animals were treated with multiple intraperitoneal injections over 14 days followed by a 14 day recovery period, emulating the clinical administration (Alsaad et al., 2012). Chronic DOX treatment in vivo caused no change in mRNA expression of the rat analogue of CYP2J2, CYP2J3. However, it increased mRNA expression of other P450 enzymes including CYP4A3, CYP4F1 and CYP4F5 known to produce the alternative AA metabolite, 20-HETE resulting in increased levels of 20-HETE compared with an untreated control group (Alsaad et al., 2012). The authors also observed a rise in the mRNA expression of the EPHX2 (sEH) gene in heart in DOX treated rats accompanied by the decreased EET levels and an increase in the levels of inactive DHETs. Furthermore, treatment of H9c2 cells, a rat cardiac cell line, with a sEH inhibitor (t-AUCB) in combination with DOX, reduced both ANP and BNP release (markers of cardiac hypertrophy), suggesting a cardioprotective effect of sEH inhibition. Additionally, DOX has been shown to upregulate levels of many P450 enzymes in this cell model including 2J3 and 2E1 (Zordoky and El-Kadi, 2008). Given the biological protective activity of EETs particularly 11,12-EET and 14,15-EET against cardiovascular disease (Sudhahar et al., 2010), it is hypothesised that EETs may provide protection against cardiotoxicity induced by a whole spectrum of xenobiotics in vivo.

Discrepancies in mRNA expression between acute and chronic DOX administration have been documented (Zordoky et al., 2010, Alsaad et al., 2012) highlighting the caution required when
interpreting studies comparing acute versus chronic DOX administration. Furthermore, the treatment period used by Alsaad may not be sufficient to induce many of the characteristic features of chronic DOX cardiotoxicity. A detailed assessment by Cove-Smith et al., 2014 of both cardiac morphology and function in rats for 8 weeks following 1.25 mg/kg DOX demonstrated changes in cardiac function, in particularly cardiac output, stroke volume and ejection fraction from day 15. Gross morphological changes and biomarkers associated with cardiomyocyte degeneration occurred much later (Cove-Smith et al., 2014). Consequently, the changes observed after 2 weeks of DOX treatment are unlikely to be a true indication of the protective role of EETs during DOX cardiotoxicity and therefore the study length would need to be extended to definitively determine this. However, it is plausible to suggest that epoxygenase enzymes are involved in the cellular development of DOX induced cardiotoxicity.

Using transgenic mice with cardiomyocyte specific overexpression of human CYP2J2, Zhang et al. (2009) identified the possible beneficial effects of EETs in the protection against DOX-induced cardiotoxicity. Elevations in serum LDH and CK and activation of cardiac caspase 3 and catalase caused by acute treatment with DOX (0, 5 and 15 mg DOX/kg/day i.p. for 3 days followed by 24 h recovery) were mitigated in mice overexpressing CYP2J2 suggesting protection against DOX induced myocardial damage. However, some of these markers are not specific to the heart and this may affect the interpretation of these findings. Following chronic treatment with DOX (0, 1.5, 3 mg/kg biweekly for 5 weeks followed by a 2 week recovery) the CYP2J2 transgenic mice showed a lower heart weight to body ratio, reduced cardiac ankyrin repeat protein (CARP) and expression ratio of βMHC: αMHC and no change in LVDP compared to wild type mice. This indicates that cardiac structure and contractile function were preserved which may have been mediated by protective EETs (Zhang et al., 2009b). Although CARP is a specific biomarker of cardiac hypertrophy, it may not be a good biomarker of early cardiac remodelling. In fact, many of the serological, pathological and
functional biomarkers of early cardiac damage during cardiotoxicity were not reported. For example, cardiac troponin which is known to be the gold standard in investigating cardiac damage, pre-clinically and clinically, was not measured (Cove-Smith et al., 2014).

Studies have found that CYP2J2 is capable of metabolising DOX (Zhang et al., 2009b). However, as this was a CYP2J2 transgenic model and not a null model other P450 enzymes may also be responsible for the increased metabolism of DOX. Furthermore, increased expression and activity of reductase in the transgenic mouse hearts may also lead to an increase in activity of many other P450 enzymes. It is known that the major metabolite of DOX, doxorubicinol (DOXol) contributes to the toxicity in the myocardium, however, there is also evidence to suggest other metabolites of DOX including DOX deoxyaglycone and DOXol hydroxyaglycone may also contribute to the cardiotoxicity (Licata et al., 2000). In addition cytochrome P450 reductase can also metabolise DOX to 7-deoxydoxyrubicin aglycone which has been shown to inhibit AA metabolism, reducing the production of EETs and altering the regiosomers of EETs produced (Arnold et al., 2017). Therefore, increased metabolism through CYP2J2 may have a conflicting effect on the heart, elevating levels of toxic metabolites for certain drugs but increasing protection through EETs. Other cytochrome P450 enzymes belonging to the murine CYP2J family, including CYP2J8, 2J11, 2J12 and 2J13, also have epoxidase activity towards AA. This may confound results especially as expression levels of these enzymes were not measured in the CYP2J2 transgenic mice (Graves et al., 2013). In rats, only CYP2J3 has been demonstrated to possess expoxyxygenase activity and so protective effects by EETs may be less apparent. In addition, the use of transgenic models where there is a genetic alteration leading to differences in protein expression and hence activity, may not directly model pharmacological activation of CYP2J2 in the wild-type mouse (Knight and Shokat, 2007).

Taken together the limited number of studies on DOX toxicity suggest CYP2J2 expression and EET production have profound cardioprotective effects, modulating DOX cardiotoxicity through their
influence on molecular pathways involved in apoptosis, ROS generation, hypertrophy and cardiac remodelling leading to an overall conservation of structure and function. It may be hypothesised that the cardiac potential of other drugs might be affected by CYP2J2 metabolism and/or EET formation. The effects of EETs on DOX raises the possibility that the CYP2J2/EET pathway may also modulate cardiotoxicity of other drugs, working through different mechanisms (table 5).

**Conclusions**

CYP2J2 is an AA and drug metabolising enzyme highly expressed in the human heart. However, the role of CYP2J2 in drug metabolism in the heart has not been well defined. Nevertheless, there is increasing awareness that many current drugs are substrates for CYP2J2. There are also substantial data suggesting that CYP2J2 along with CYP3A4 plays a significant part in the metabolism of drugs known to cause cardiotoxicity. Therefore, changes in the expression or activity of this enzyme may alter drug concentrations in the body leading to either an ineffective drug response or increased levels of metabolites leading to potential cardiotoxicity. Known P450 inducers do not modulate CYP2J2 levels but some compounds have been recognised to induce expression and activity of CYP2J2 in adult human cardiomyocytes. In addition, several SNPs in the human CYP2J2 gene are associated with altered *in vitro* activity of the CYP2J2 enzyme, which may lead to changes in EET formation and drug metabolism, potentially altering cardiotoxicity in some individuals. As well as its role in drug metabolism, CYP2J2 derived EETs have also been shown to have a protective effect, although this has only really been reported for DOX-induced cardiotoxicity. Given that our understanding of the role of CYP2J2 and EET formation is largely based on the data from this single drug and the importance of cardiotoxicity to drug development and application in man, understanding more widely the role and possible protection by CYP2J2 in the heart is worthy of further study.
Authorship contribution

Wrote or contributed to the writing of the manuscript: Solanki, Pointon, Jones and Herbert.
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Footnote

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Legends to figures

Figure 1 Overview of hypothesised CYP2J2 functions in the heart. CYP2J2 has a role in drug metabolism in the heart which may lead to either detoxification of drugs or cardiotoxicity. This could be counteracted by its epoxide activity by which it produces several protective molecules including EETs. Doxorubicin inhibits production of EETs through inhibiting epoxygenase enzymes and increasing sEH mRNA production in rats. However addition of exogenous EETs protects against DOX toxicity in H9c2 cells (Zhang et al., 2009b). It may be proposed that the CYP2J2/EET pathway has a role in protecting against other drug-related toxicities within the heart. Furthermore, although there is little evidence regarding drug-AA interactions, the competition between drug substrates and AA may alter the balance of protective EETs and cardiotoxic compounds. Drugs such as dronedarone have been shown to inhibit CYP2J2 in a non-competitive way and so inhibit EET formation in an in vitro system (Karkhanis et al., 2016, Karkhanis et al., 2017).

Figure 2 Relative mRNA expression of CYPs in human cardiomyocytes and human heart tissue and cardiomyocytes. Total RNA was extracted and reverse-transcription polymerase chain reaction (RT-PCR) was carried out. The house keeper GusB was used a housekeeper gene. $2^{\Delta CT}$ calculation was used to quantitate CYP2J2 mRNA expression. 10 P450 enzymes were investigated of which CYP2J2 had the highest expression. Reproduced with permission from DMD (Evangelista et al., 2013).

Figure 3 The formation of EETs by CYP2J2. AA is hydrolysed from the phospholipid bilayer and is the precursor for eicosanoids including prostaglandins, Leukotrienes and HETEs. Epoxidation by CYP2J2 leads to production of 4 different EETs which can be deactivated by sEH to form DHETs.

Figure 4 Chromatographic data showing the differences in products formed by recombinant CYP3A4 and CYP2J2. Albendazole metabolism by both CYP3A4 and CYP2J2 produced the same metabolite, however an additional metabolite was observed for CYP2J2 indicating hydroxylation. CYP2J2 and CYP3A4 produced similar peaks indicating hydroxylation. Chromatographic data of astemizole metabolism showed CYP3A4 produced the hydroxylated product whereas metabolism by CYP2J2 resulted in both the o-dealkylated metabolite and some N-dealkylated product. When Danazol was incubated with both enzymes similar metabolites were produced. Reproduced with permission from DMD (Lee et al., 2010).
### Table 1

Table 1: Amino acid sequence homology between human CYP2J2 and mammalian CYP2Js. Humans, monkey and dog all have single isoforms for CYP2J2 whereas rodents such as rat have multiple isoforms, CYP2J3 and 2J4 being the most similar to human. Values are percent sequence homology. (Reproduced from permission from DMD)

|            | Human 2J2 | Monkey 2J2 | Dog 2J2 | Rat 2J3 | Rat 2J4 |
|------------|-----------|------------|---------|---------|---------|
| Human 2J2  | 100       | 95         | 79      | 72      | 76      |
| Monkey 2J2 | 100       | 81         | 74      | 76      |         |
| Dog 2J2    | 100       |            | 72      |         |         |
| Rat 2J3    |           |            | 100     | 79      |         |
| Rat 2J4    |           |            |         |         | 100     |
Table 2: CYP2J gene expressions in human and other mammalian tissues. Expression is quantified, whereby (+) is low expression, (++) is moderate expression and (+++) is high expression. Within the heart human CYP2J2 has the highest RNA and protein expression. Rat CYP2J3 is expressed highly at the protein level and mouse CYP2J11 has high RNA expression in the heart indicating that there are often discrepancies between RNA and protein expression in different homologues. (Adapted from Xu et al., 2013).

| Tissue          | Human | Mouse | Monkey | Rat |
|-----------------|-------|-------|--------|-----|
|                 | 2J2   | 2J5   | 2J6    | 2J8 | 2J9 | 2J11 | 2J12 | 2J13 | 2J2 | 2J3 | 2J4 |
| Liver           | RNA   |       |        |      |      |      |      |      |     |     |     |
|                 | Protein | ++ | +   | ++ | +   | ++ | ++ | +++ | ++ | ++ | ++ |
| Heart           | RNA   | +++  |      |      |      |      |      |      |     |     |     |
|                 | Protein | +++ | +   |      | +++ |      | +   | +   |      |     |     |
| Small intestine | RNA   | ++   |      | +++ |      | ++ | +   | +   |     |     |     |
|                 | Protein | +   |      |      | +   | +   | +   | +   | +++ | +++ | +++ |
| Lung            | RNA   | +    |      |      |      |      |      |      | +   | +   | +   |
|                 | Protein | +   |      |      |      | +   |      |      | +   | +   | +   |
| Kidney          | RNA   | +    | +++  |      | ++ | +++ |      |      |      |     |     |
|                 | Protein | ++  | +    | +++ | ++ | +++ | +++ |      |     |     |     |
| Brain           | RNA   | +    |      | ++ | +   | +++ |      |      |      |     |     |
|                 | Protein | +   |      | ++ | +++ |      |      |      |     |     |     |
| Pancreas        | RNA   | +    |      |      |      |      |      |      |     |     |     |
|                 | Protein | +   |      |      |      |      |      |      |     |     |     |
| Stomach         | RNA   |      |      |      |      |      |      |      | +   | +   | +   |
|                 | Protein |      |      |      |      |      |      |      |      |     |     |
| Spleen          | RNA   |      |      |      |      |      |      |      | +   | +   | +   |
|                 | Protein |      |      |      |      |      |      |      |     |     |     |
| Skeletal muscle | RNA   | ++   |      |      |      |      |      |      |     |     |     |
|                 | Protein |      |      |      |      |      |      |      |     |     |     |
Table 3: CYP2J2 allelic variations in humans known to have changes in activity in vitro. Currently only a variation in the CYP2J2*7 allele has been shown to have associations with disease. AA (arachidonic acid) LA (linoleic acid). Adapted from: (Berlin et al., 2011).

| Allele  | cDNA/Gene | Enzyme activity in vitro | Association with disease | References |
|---------|-----------|--------------------------|--------------------------|------------|
| CYP2J2*2 | 427A>G    | Reduced AA and LA metabolism | No known association with disease | (King et al., 2002) |
| CYP2J2*3 | 472C>T    | Reduced AA and LA metabolism | No known association with disease | (King et al., 2002) |
| CYP2J2*4 | 575T>A    | Reduced AA metabolism    | No known association with disease | (King et al., 2002) |
| CYP2J2*5 | 1024G>A   | Produced wild type levels of AA and LA metabolites | No known association with disease | (King et al., 2002) |
| CYP2J2*6 | 1210A>T   | Reduced AA and LA metabolism | No known association with disease | (King et al., 2002) |
| CYP2J2*7 | -50G>T    | Reduced transcription due to loss of Sp1 binding site | Type 2 diabetes, Alzheimer’s disease, coronary artery disease, Ischaemic stroke | (King et al., 2002, Spiecker et al., 2004, Wang et al., 2010, Yan et al., 2015, Zhu et al., 2013, Wang et al., 2017) |
| CYP2J2*8 | 934G>A    | Complete loss of enzymatic activity | No known association with disease | (Lee et al., 2005) |
| CYP2J2*9 | 1052C>T   | Enzymatic activity comparable to wild type | No known association with disease | (Lee et al., 2005) |
| CYP2J2*10 | 344C>T    | Reduced function protein | No known association with disease | (Gaedigk et al., 2006) |
Table 4

Table 4 The range of substrates for CYP2J2 and the metabolic pathways by which they are formed. Substrates include both endogenous compounds (a) and exogenous drugs (b) and the main metabolic pathways are hydroxylation and epoxygenation. Some $K_m$ and $V_{max}$ values remain unknown and this is indicated by shaded cells. * Metabolic pathway used solely by CYP2J2.

| Substrate               | Metabolic pathway          | $K_m$ (μM) | $V_{max}$/turnover number (nmol/min/nmol) |
|-------------------------|----------------------------|------------|----------------------------------------|
| a) Endogenous           |                            |            |                                        |
| Arachidonic acid        | Epoxygenation              |            |                                        |
| Linoleic acid           | Epoxygenation              | 0.105      |                                        |
| Docosahexaenoic acid    | Epoxygenation (major)      |            |                                        |
|                         | $\omega-1/\omega$ Hydroxylation (minor) |            |                                        |
| Eicosapentaenoic acid   | Epoxygenation (major)      |            |                                        |
|                         | $\omega-1/\omega$ Hydroxylation (minor) |            |                                        |
| Vitamin D3              | 25-Hydroxylation           | 7.7±1.2    | 0.087±0.013                            |
| Vitamin D2              | 25-Hydroxylation           | 2.0±0.3    | 0.16±0.03                              |
| 1α(OH)D3                | 25-Hydroxylation           | 4.4±0.7    | 2.2                                    |

b) Drugs

| Substrate               | Metabolic pathway          | $K_m$ (μM) | $V_{max}$/turnover number (nmol/min/nmol) |
|-------------------------|----------------------------|------------|----------------------------------------|
| Albendazole             | $\omega$-Hydroxylation     |            |                                        |
|                         | Sulfoxidation              |            |                                        |
| Amiodarone              | 3-Hydroxylation            | 5          | 4.6                                    |
|                         | 4-Hydroxylation            |            |                                        |
| Apixaban                | O-demethylation            |            | 0.27±0.06                              |
| Astemizole *            | O-demethylation            | 0.65       | 1.129                                  |
| Benzphetamine           | N-demethylation            |            | 0.08                                   |
| Bufuralol               |                            |            | 0.17                                   |
| Cyclosporine A          | Hydroxylation              |            |                                        |
| Danazol                 | Hydroxylation              |            |                                        |
| Ebastin *               | Hydroxylation              | 1.3/18.3   | 40.6/8.2                               |
| Eperisone               | $\omega$-Hydroxylation     |            | 0.0266                                 |
|                         | $\omega-1$ Hydroxylation   |            |                                        |
| Hydroxyebastine         | Carboxylation              | 0.75       | 9.86                                   |
| Terfenadine             | Hydroxylation              | 0.4        | 20                                     |
| Thioridazine            | Sulfoxidation              |            |                                        |
| Vorapaxar               | Hydroxylation              |            | 0.0306                                 |
| CYP2J2 substrate | FDA approval package cardiac warnings | Mechanism contributing to cardiotoxicity | Circumstantial evidence for mitigation of cardiotoxicity by EETs |
|------------------|--------------------------------------|------------------------------------------|-------------------------------------------------------------|
| cyclosporine A (Tang et al., 2011) | Hypertension, tachycardia, myocardial infarction | Oxidative stress and/or mitochondrial dysfunction | In a CYP2J2 transgenic mouse, heart failure-induced oxidative stress was mitigated by EETs; EETs increased expression of antioxidant enzymes and reduced reactive oxygen species levels (Wang et al., 2014, Akhnokh et al., 2016). |
| doxorubicin (Zhang et al., 2009b) | ECG abnormalities, tachyarrhythmias, reduction in LVEF and congestive heart failure | | In a CYP2J2 transgenic mouse, doxorubicin-induced ROS levels were reduced compared with wild type. CYP2J2 transgenic mice had preserved mitochondrial structure and membrane potential (Zhang et al., 2009b). |
| sunitinib (Aparicio-Gallego et al., 2011) | Decreased LVEF and HF, QT interval prolongation and TdP, cardiomyopathy. | | In E3EH null mice, increased circulating EETs limited mitochondrial damage following ischaemia (Akhnokh et al., 2016). |
| amiodarone (Isomoto et al., 2006) | Ventricular fibrillation, ventricular tachycardia, QTc prolongation. | Activation of apoptotic pathways and caspases | EETs inhibited pro-apoptotic pathways through increasing activity of the pro-survival enzyme phosphoinositide 3 kinase (PI3K) in mouse primary cardiomyocytes (Dhanasekaran et al., 2008). |
| sunitinib (Aparicio-Gallego et al., 2011) | As above | | |
| astemizole (Minotti, 2010) | QTc interval prolongation in a dose related manner. Cardiac dysrhythmia. | Changes in electrophysiology | No evidence to date |
| terfenadine (Minotti, 2010) | | | |
| thioridazine (Minotti, 2010, Menkes and Knight, 2002) | | | |
| sunitinib (Aparicio-Gallego et al., 2011) | As above. | Cardiac hypertrophy | In an animal model of isoproterenol-induced cardiac hypertrophy, use of sEH inhibitors protected in rats (Althurwi et al., 2013). |
| 5-fluorouracil (Alter et al., 2006) | Angina, myocardial infarction, arrhythmia and heart failure. | Vasoconstriction | 11,12-EET caused relaxation of rat coronary arteries and renal and cerebral arteries of rats and rabbits. (Larsen et
| Drug                  | Effect                                      | Mechanism                                                                 | Evidence         |
|----------------------|---------------------------------------------|---------------------------------------------------------------------------|------------------|
| cyclosporine A       | As above.                                   | Increase in intracellular calcium concentration through the calcium sensing receptor (CaSR) | No evidence to date |
| eperisone            | QTc interval prolongation                   | Inhibition of nicotinic and muscarinic receptors                            | No evidence to date |

References:
- Rezzani et al., 2005
- Yamagiwa et al., 2014, 1991
- Saegusa et al., 1991
Figure 1
Figure 2

[Graph showing relative mRNA levels for human cardiomyocytes and human heart for various CYP enzymes.]
Figure 3
Figure 4

Products of CYP3A4

- Abendazole
- Amiodarone
- Astemizole
- Danazol

Products of CYP2J2

- Abendazole
- Amiodarone
- Astemizole
- Danazol