2017

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BCP-D-17-00116

Commentary

Inhibition and Inactivation of Human CYP2J2: Implications in Cardiac Pathophysiology and Opportunities in Cancer Therapy

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ABBREVIATIONS

2-arachidonylglycerol	2-AG
17-octadecynoic acid	17-ODYA
action potential duration	APD
arachidonic acid	AA
arachidonyl ethanolamide	AEA
calcium-dependent potassium channels	BK_{ca}
CYP2J2-TG	CYP2J2 transgenic
cytochrome P450 enzyme	CYP450
dihydroxyeicosatrienoic acids	DHETs
docosahexaenoic acid	DHA
eicosapentaenoic acid	EPA
endothelial-derived hyperpolarising factors	EDHF
epoxydocosapentaenoic acids	EDPs
epoxyeicosatetraenoic acids	EEQs
epoxyeicosatrienoic acid ethanolamide	EET-EA
epoxyeicosatrienoic acids	EETs
epoxyoctadecenoic acids	EOAs
hydroxyeicosatetraenoic acid ethanolamide	HETE-EA
linoleic acid	LA
mechanism-based inactivation	MBI
metabolite-intermediate complex	MI complex
microRNAs	miRNAs
mitochondrial ATP-dependent potassium channels	$mitoK_{ATP}$
mitogen-activated protein kinase	MAPK
N-desbutyldronedarone	NDBD
N-desethylamiodarone	NDEA
nuclear factor kappa-light-chain-enhancer of activated B cells	NF-κB
17-octadeynoic acid	17-ODYA
peroxisome proliferator-activated receptor gamma	PPARγ
phosphatidylinositide 3-kinase	PI3K
polyunsaturated fatty acids	PUFAs
sarcoplasmic/endoplasmic reticulum calcium ATPase	SERCA2a
soluble epoxide hydrolase	sEH
tumor necrosis factor alpha	TNFα
tyrosine kinase inhibitors	TKIs
matrix metalloproteinases	MMP

BCP-D-17-00116

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Number of Tables	4
Number of References	101
Number of Words in the Abstract	177

BCP-D-17-00116

ABSTRACT

Extrahepatic cytochrome P450 enzymes (CYP450) are pivotal in the metabolism of endogenous substrates and xenobiotics. CYP2J2 is a major cardiac CYP450 and primarily metabolizes polyunsaturated fatty acids such as arachidonic acid to cardioactive epoxyeicosatrienoic acids. Due to its role in endobiotic metabolism, CYP2J2 has been actively studied in recent years with the focus on its biological functions in cardiac pathophysiology. Additionally, CYP2J2 metabolizes a number of xenobiotics such as astemizole and terfenadine and is potently inhibited by danazol and telmisartan. Notably, CYP2J2 is found to be upregulated in multiple cancers. Hence a number of specific CYP2J2 inhibitors have been developed and their efficacy in inhibiting tumor progression has been actively studied. CYP2J2 inhibitor such as C26 (1-[4-(vinyl) phenyl]-4-[4-(diphenyl-hydroxymethyl)-piperidinyl]-butanone hydrochloride) caused marked reduction in tumor proliferation and migration as well as promoted apoptosis in cancer cells. In this review, we discuss the role of CYP2J2 in cardiac pathophysiology and cancer therapeutics. Additionally, we provide an update on the substrates, reversible inhibitors and irreversible inhibitors of CYP2J2. Finally, we discuss the current gaps and future directions in CYP2J2 research.

Keywords: CYP2J2, arachidonic acid, epoxyeicosatrienoic acids; cardiology; oncology

1. Introduction

Extrahepatic cytochrome P450 enzymes (CYP450) play a dominant role in xenobiotic metabolism and organ-specific toxicity [1]. For example, skatole or 3-methylindole, that is derived from the colonic hydration of tryptophan and present in cigarette smoke, is dehydrogenated by lung-specific CYP2F1 to highly reactive 3-methyleneindolenine [2]. This reactive metabolite intercalates with DNA and is cytotoxic to bronchial epithelial cells in humans [3]. CYP2J2 is a CYP450 expressed predominantly in the heart [4] although it has been measured in liver [5], gastrointestinal tract [6], pancreas [7], lungs [8], brain [9] and other tissues. CYP2J2 is the only isoform of CYP2J family found in humans. CYP2J2 is primarily found in cardiomyocytes, coronary arterial endothelial cells and to a lesser extent in vascular smooth muscles cells and aorta [10,11]. Compared to the atria, the ventricles have higher expression of CYP2J2 mRNA, while the levels are equal between the two ventricles [12]. CYP2J2 metabolizes endogenous polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA) [13] and linoleic acid (LA) [14] as well as xenobiotics such as terfenadine [15] and astemizole [16]. CYP2J2 has garnered increased attention in recent years as it was discovered to be upregulated in haematological malignancies [17] and certain carcinomas [18]. This has led to an explosion of new research in the field of cancer therapeutics with novel CYP2J2 inhibitors. Since CYP2J2 enzyme straddles between endobiotic and xenobiotic metabolic pathways, researchers are evaluating the perturbation of its endobiotic pathways due to CYP2J2 inhibition or induction.

Like CYP2D6, a number of single nucleotide polymorphisms of CYP2J2 have been discovered. CYP2J2*7 is the most commonly found SNP with marginally reduced CYP2J2 expression while CYP2J2*8 (rs150461093) polymorphism (heterozygote) exhibits complete loss of metabolic activity. Although CYP2J2*7 has been associated with incidence of hypertension in Russian and Saudi populations, strong correlation between CYP2J2*7 and

hypertension across gender and race has not been established. Similarly, no significant correlation between CYP2J2*7 and coronary artery disease is proven. It is currently unknown whether carriers of other CYP2J2 polymorphisms are predisposed to cardiovascular diseases. CYP2J2 polymorphisms and their implications in cardiovascular diseases such as hypertension, coronary artery disease and myocardial infarction have been comprehensively reviewed [19].

In this commentary, we first discuss the substrates, inhibitors, and mode of inhibition of CYP2J2. Secondly, we present biological roles of CYP2J2 and its AA metabolites, along with the implications of CYP2J2 inhibition in cardiac pathophysiology. Thirdly, we discuss the role and consideration of CYP2J2 inhibitors in cancer therapeutics. Lastly, we highlight the current gaps in CYP2J2 research and provide directions for future research.

2. Substrate and inhibition of CYP2J2

2.1 Endogenous substrates of CYP2J2

CYP2J2 is an epoxygenase enzyme metabolizing a number of polyunsaturated omega-6 (ω-6) fatty acids such AA and LA and omega-3 (ω-3) fatty acids such as eicosapentaenoic acid (EPA) [20] and docosahexaenoic acid (DHA) [21]. CYP2J2 metabolizes AA to regioisomeric and stereoselective epoxyeicosatrienoic acids (EETs) namely 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET; LA is converted to epoxyoctadecenoic acids (EOAs) such as 12,13-EOA and 9,10-EOA; EPA forms epoxyeicosatetraenoic acids (EEQs) such as 17,18-EEQ and DHA yields epoxydocosapentaenoic acids (EDPs) such as 19,20-EDP (Table 1) [21,22]. CYP2J2-mediated metabolism of AA has garnered a lot of attention in recent years owing to the cardioprotective roles of EETs. Nevertheless, it is noteworthy that the metabolic turnover rates for CYP2J2 for EPA (0.943 nmol/min/nmol CYP2J2) and DHA (0.228 nmol/min/nmol CYP2J2) are higher than LA (0.105 nmol/min/nmol CYP2J2) and AA (0.065 nmol/min/nmol CYP2J2) [20,22,23]. This reflects the different intrinsic clearances of the endogenous

substrates by CYP2J2. Recently, Arnold *et al*. performed extensive studies to determine the Michaelis-Menten kinetic parameters of CYP2J2-mediated metabolism of the polyunsaturated fatty acids (PUFAs) [24]. The K_m value for AA metabolism was found to be 131 µM while that for xenobiotics such as astemizole and terfenadine are 0.65 µM and 0.4 µM respectively. This difference in the substrate affinities (K_m values) between endogenous and exogenous substrates suggests that CYP2J2 may preferentially metabolize xenobiotics in the presence of endogenous substrates. Considering CYP2J2 is an extrahepatic CYP450 and heart is not the main xenobiotic elimination organ, this knowledge is clinically insightful. Apart from CYP2J2, CYP2C8 and CYP2C9 are involved in EET biosynthesis in the heart and this information has been reviewed previously [25]. It is noteworthy that EETs are readily metabolized by soluble epoxide hydrolase (sEH) to their respective regioisomeric vicinal diols, dihydroxyeicosatrienoic acids (DHETs) [26]. DHETs exhibit similar physiological actions as EETs but are significantly less potent compared to EETs [27]. Besides PUFAs, CYP2J2 metabolizes vitamin D2 and D3 to 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 respectively [28]. Since CYP2J2 expression in skin and liver is limited, the physiological implication of CYP2J2-mediated bioactivation of vitamin D needs further investigation. Lastly, the perturbation of the endocannabinoid system is linked to cardiovascular, gastrointestinal and immunological disorders [29]. McDougle *et al.* discovered that two endocannabinoids namely arachidonyl ethanolamide (AEA) and 2-arachidonylglycerol (2-AG) undergo metabolism by CYP2J2 to form predominantly epoxyeicosatrienoic ethanolamide (e.g. 14,15-EET-EA) and epoxyeicosatrienoic glycerol (e.g. 14,15-EET-G) [30]. In a similar study, other metabolites of AEA such as 11,12-EET-EA, 8,9-EET-EA, 5,6-EET-EA, 20-hydroxyeicosatetraenoic ethanolamide (20-HETE-EA) and 19-HETE-EA were determined in human intestinal microsomes [31]. It was also shown that AA was a moderate inhibitor of CYP2J2-mediated metabolism of AEA [31]. The authors found that 5,6-EET-EA is a potent cannabinoid receptor-

2 agonist. Activation of cannabinoid receptor-2 leads to anti-inflammatory response. Consequently, the authors postulate that inhibition of AEA and 2-AG metabolism may be linked to gastrointestinal disorders such as inflammatory bowel syndrome.

2.2 Exogenous substrates of CYP2J2

Besides astemizole and terfenadine, Lee *et al.* determined that tamoxifen, mesoridazine, and thioridazine are rapidly metabolized by CYP2J2 [32]. Non-Vitamin K antagonist oral anticoagulants (NOACs) such as apixaban and rivaroxaban are reported to be metabolized by CYP2J2 [33,34]. Vorapaxar, a novel protease-activated receptor-1 (PAR-1) antagonist and organo-nitrates such as NO-aspirin, isosorbide dinitrate, nitroglycerin also undergo rapid metabolism by CYP2J2 [35]. Metabolism of these drugs by CYP2J2 may eventually reduce their intracardiac concentrations and affect other pharmacokinetic parameters leading to sub-therapeutic outcomes.

A homology model of CYP2J2 active site developed using CYP2A6, CYP2B4, CYP2C8, CYP2C9 and CYP2D6 as templates revealed that CYP2J2 has a restrictive active site due to the presence of bulky amino acid residues such as I127, F310, A311, T315, I375, I376 and V380 in proximity to the heme group [36]. The model also revealed that this hydrophobic pocket is the only available access for substrates to CYP2J2 heme group. It was further discovered that the shorter the distance (in angstrom) between the CYP2J2 heme iron and the carbon atom on the terminal aliphatic hydrocarbon chain, the greater is the extent of hydroxylation. This model highlights that CYP2J2-specific metabolites are generated by hydroxylation of the least sterically hindered chemical moieties on the molecule. For example, albendazole and amiodarone are metabolized specifically by CYP2J2 to ω-hydroxyalbendazole and 4-hydroxyamiodarone respectively [32,37]. It is notable that CYP2J2-specific hydroxylation occurs on the terminal carbon atom along the respective propyl and butyl chains

of albendazole and amiodarone. This corroborates the observed narrow and restrictive active site within the CYP2J2 homology model. With the identification of CYP2J2-specific metabolites, the contribution of CYP2J2 to the overall metabolic clearance of these drugs can be predicted using various static and dynamic modeling techniques.

2.3 Substrate inhibition

A number of CYP2J2 substrates exhibit non-classical Michaelis-Menten kinetics. At higher substrate concentrations, substrate inhibition or auto-inhibition of CYP2J2 is observed [38]. It is unclear if such modes of inhibition occur at the active or allosteric binding site of CYP2J2. The latter binding site has not been demonstrated thus far due to the lack of CYP2J2 crystal structure and is also not represented within the CYP2J2 homology models. Drugs such as albendazole, cyclophosphamide, dronedarone and ebastine exhibit substrate inhibition of CYP2J2 [39–42].

2.4 Reversible inhibition of CYP2J2

Using astemizole and terfenadine as probe substrates, a number of marketed drugs have been identified as CYP2J2 reversible inhibitors [38]. Notably, many of the substrates of CYP2J2 are reversible inhibitors of CYP2J2 (e.g. amiodarone, astemizole, danazol, tamoxifen and terfenadine). Among these drugs, danazol is the most potent reversible inhibitor of CYP2J2 [37]. On the other hand, telmisartan and flunarizine were found to be non-substrate inhibitors of CYP2J2. Our laboratory previously reported dronedarone, amiodarone and their two respective major metabolites namely *N*-desbutyldronedarone (NDBD) and *N*-desethylamiodarone (NDEA) [44,45], are potent reversible inhibitors of CYP2J2 with dronedarone showing comparable inhibitory potency (K_i = 34 nM) to danazol (K_i = 20 nM) [39]. Dronedarone and NDBD exhibit a mixed mode while amiodarone and NDEA exhibits

non-competitive mode of inhibition of CYP2J2 [39]. This observation hints the presence of an allosteric binding site on CYP2J2. In due course, other groups have identified a number of natural compounds such as decursin and tanshinone IIA as potent reversible inhibitors against CYP2J2-mediated astemizole *O*-demethylation [46,47]. The relative potencies of CYP2J2 inhibitors have been compared and reported previously [19]. Intriguingly, Arnold *et al*. demonstrated that PUFAs such as AA, LA, EPA and DHA inhibit the CYP2J2-mediated metabolism of other PUFAs [24]. For instance, DHA inhibits AA metabolism at a K_i of 16.5 μM while AA inhibits DHA epoxidation at a K_i of 65.2 μM. This finding sheds new light on the metabolic interactions between PUFAs and their potential to interfere with their intrinsic clearances.

Evangelista *et al*. reported the extent of CYP2J2 inhibition in human primary cardiomyocytes [48]. Notably, there are observed similarities and differences between the potencies of inhibitors when tested against recombinant versus cellular CYP2J2. For instance, danazol and cisapride are potent inhibitors against both recombinant and cardiomyocyte CYP2J2 while amiodarone is equally less potent in either enzyme model. On the other hand, lansoprazole inhibited recombinant CYP2J2 potently but not cardiomyocyte CYP2J2, while astemizole inhibits CYP2J2 potently in human cardiomyocytes as compared to recombinant CYP2J2 [49]. Such observed different inhibitory potencies of enzymatic versus cellular CYP2J2 might be due to several underlying factors that need to be addressed. These include differential enzymatic activities associated with recombinant versus native CYP2J2 and different fraction unbound ($f_{u,incubation}$) of inhibitors in each protein-rich *in vitro* system.

2.5 Irreversible inhibition of CYP2J2

Lafite *et al*. synthesized terfenadine derivatives as selective CYP2J2 inhibitors and found compounds 5 and 13 exhibited mechanism-based inactivation (MBI) of CYP2J2, with

compound 13 forming a stable Fe^{2+}-carbene metabolite-intermediate (MI) complex [50]. Compounds 5 and 13 are the earliest examples of mechanism-based inactivators of CYP2J2 (Table 2). Subsequently, ritonavir was identified as a potent mechanism-based inactivator of CYP2J2 [51] while our laboratory reported similar observation for dronedarone, amiodarone and NDBD [39]. Dronedarone and NDBD form distinct quinone-oxime reactive metabolites that are adducted covalently to CYP2J2 protein. While 17α-ethinyl estradiol exhibits weak time-dependent inhibition of recombinant CYP2J2 based on IC_{50} shift assay [52], its irreversible MBI of CYP2J2 needs to be further confirmed.

3. CYP2J2 in cardiac pathophysiology

3.1 Biological functions of EETs

The biological functions of EETs have been widely studied using various cellular and animal models and extensively reviewed [53–55] . In this section, we aim to provide a concise summary of the functions of EETs comprising of vasodilation, anti-inflammation, angiogenesis, anti-apoptosis and ion-channel modulation.

EETs are primarily endothelial-derived hyperpolarising factors (EDHF) bringing about vasodilation independent of nitrous oxide or prostacyclin formation. Campbell *et al.* first discovered that EETs acted as EDHFs by activating large-conductance calcium-dependent potassium channels (BK_{ca}) in bovine coronary arterial smooth muscle cells [56] and this was subsequently proven in human coronary arterioles [57]. Campbell *et al.* found that all four regioisomers are equipotent vasodilators while Larsen *et al.* discovered 14,15-EET was least potent of all four regioisomers [57]. EETs bring about vasodilation mainly via three known mechanisms [58] namely (1) activation of the transient receptor potential cation channel subfamily V member 4 (TRVP4) Ca^{2+} channel followed by activation of rynanodine receptors on sarcoplasmic reticulum leading to vasorelaxation in vascular smooth muscle cells, (2)

activation of the transient receptor potential channels namely TRPC3 and TRPC6 resulting in Ca^{2+} influx in endothelial cells, activation of small-conductance calcium-dependent potassium channels and intermediate-conductance calcium-dependent potassium channels, and eventually membrane hyperpolarization, and (3) activation of the cAMP/PKA pathway and BK_{Ca} channels and subsequent smooth muscle cell membrane hyperpolarization.

The anti-inflammatory effects of EETs were first disocverd in a landmark study by Node *et al.* where it was found that 11,12-EET decreases the mononuclear cell adhesion and increases mononuclear cell rolling in tumor necrosis factor alpha (TNFα)-induced injury in mice carotid arteries [11]. EETs produce anti-inflammatory effect on endothelium by inhibiting the transcription of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) and degradation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha, IκBα [11]. 11,12-EET specifically attenuates TNFα-induced NF-κB activation in umbilical cord endothelial cells as well as reduces the expression of cell adhesion proteins vascular cell adhesion protein 1 and intercellular adhesion molecule 1 leading to suppression of NF-κB expression [11]. 11,12-EET, 8,9-EET and 5,6-EET demonstrate anti-inflammatory effects (strongest to weakest). Unexpectedly, 14,15-EET does not show anti-inflammatory effect.

EETs stimulate angiogenesis via three major pathways namely (1) activation of the cAMP response-element binding protein and cyclooxygenase-2 expression via cAMP/PKA pathway [59], (2) increased expression of cyclin D1 via phosphatidylinositide 3-kinase (PI3K)/Akt pathway [60], and (3) activation of p38 mitogen-activated protein kinase (MAPK) pathway activated specifically by 8,9-EET and 11,12-EET [61]. The degree of angiogenesis and the activation of a specific pathway depend on the type of endothelium, EET regioisomer and whether it is the direct action of EETs or angiogenesis being a secondary response. It has also been noted that EETs elicit anti-apoptotic activity in endothelial cells by inhibition of

MAPK dephosphorylation and PI3K/Akt activation [62]. On the other hand, 8,9-EET protect pulmonary artery smooth muscle cells from serum deprivation-induced cell death in part via Rho-kinase pathway [63].

Last but not least, EETs are known to modulate cardiac ion channels such as ATP sensitive-K^+ (K_{ATP}), L-type Ca^{2+} and Na^+ channels. 11,12-EET enhanced L-type Ca^{2+} currents via cAMP/PKA system [64] and activated the K_{ATP} channels by reducing the channel sensitivity to ATP in isolated rat cardiomyocytes [65]. 8,9 EET inhibits cardiac sodium channels in isolated rat cardiomyocytes [66]. Activation of mitochondrial K_{ATP} (mitoK_{ATP}) channels protects the heart against myocardial ischemia-reperfusion injury, suggesting that myocardial preconditioning may occur by the direct action between EETs and the channels.

3.2 Role of CYP2J2 in cardiac pathophysiology

As EETs are associated with cardioprotective and vasoprotective functions, there is an increasing impetus to investigate the role of CYP2J2 in cardiac pathophysiology. Seubert *et al.* developed CYP2J2 transgenic (CYP2J2-TG) mice with cardiomyocyte-specific overexpression of CYP2J2 [67]. This transgenic mouse model has been extensively used to study the effect of cardiac specific CYP2J2 overexpression in numerous physiological and pathophysiological conditions by different research groups. Seubert *et al*. reported that the hearts of CYP2J2-TG mice were anatomically and physiologically similar to wild-type (WT) mice but had increased capacity for EET biosynthesis. The transgenic mice exhibited an improved recovery of the left ventricular function from post-ischemic shock. This effect was attributed to the activation of mitoK_{ATP} channels and p42/p44 MAPK pathway [67]. One of the significant differences between CYP2J2-TG and WT mice is the ability to recuperate after a cardiac insult. Westphal *et al.* demonstrated that CYP2J2-TG mice were less susceptible to transverse aortic constriction or β-adrenergic stimulation induced maladaptive cardiac hypertrophy. CYP2J2-TG

mice did not exhibit ventricular tachyarrhythmia post hypertrophic insult and were one seventh as likely to succumb to the insult. CYP2J2-TG mice also showed lower atrial refractoriness, atrial fibrosis formation and susceptibility to atrial fibrillation, characteristics of cardiac hypertrophy. These effects have been attributed to the increased synthesis of EETs, delocalization of ventricular connexion-43 and activation of mitoK_{ATP} channels [68]. In a separate study, CYP2J2-TG mice showed evidence of reduced angiotensin-II induced endoplasmic reticulum stress, cardiomyocyte apoptosis, intracellular Ca^{2+} overload, intracellular reactive oxygen species (ROS) and prevention of loss of sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a) expression. Similar phenotypic changes were observed after treatment with exogenous EETs confirming that the cardioprotective effects CYP2J2 overexpression are due to elevated intracardiac EET levels [69]. Similarly, CYP2J2 overexpression mitigated the inflammatory response in cardiomyocytes in angiotensin-II-induced cardiac fibrosis [70]. . Moreover, it has been previously reported that CYP2J2 and other cardiac CYP450 are upregulated in cardiac failure possibly to compensate for dysregulated cardiac homeostasis [71]. Since cardiac hypertrophy and heart failure are associated with multiple pathological conditions like cardiac fibrosis [72], altered calcium handling [73], heightened inflammatory responses and arrythmogenesis [74]. it renders management of heart failure a herculean challenge. In summary, CYP2J2 overexpression may open new avenues in the management of complex cardiovascular diseases such as heart failure by simultaneously targeting multiple pathological conditions

Doxorubicin is a known cardio toxicant acting via myriad molecular pathways such as mitochondrial damage, intracellular calcium dysregulation, weakening of extracellular collageneous matrix due to increased formation of matrix metalloproteinase-2 (MMP-2) and MMP-9, ROS and ceramide triggered apoptosis of cardiomyocytes [75]. Upon acute and chronic treatment of CYP2J2-TG mice with doxorubicin, increase in serum lactate

dehydrogenase and creatine kinase levels was observed and consistent with cellular damage. Notably, the observed response in CYP2J2-TG mice was half that of WT mice [76]. Additionally, CYP2J2-TG mice yielded less activation of oxidative stress enzyme like catalase (2.5 fold) and apoptotic marker like caspase-3 (1.4 fold) and less mitochondrial damage in the cardiomyocytes when compared to WT. It was further observed that the *in vitro* pretreatment of rat cardiomyocyte H9c2 cells with 11,12-EET attenuated doxorubicin-induced mitochondrial fission and loss of mitochondrial membrane integrity. The cardioprotective effects of CYP2J2 overexpression was attributed not only to the elevated levels of EETs, but also to the relatively rapid intrinsic clearance of doxorubicin as measured using heart microsomes isolated from CYP2J2-TG mice. Although the researchers did not explore the effect on superoxide dismutase activity, ceramide accumulation and extracellular matrix remodelling, their seminal work sheds light on the cardioprotective effects of CYP2J2 overexpression that in turn hints at a novel therapeutic strategy to combat drug-induced cardiotoxicity.

Diabetic cardiomyopathy is characterized by left ventricular hypertrophy, interstilial fibrosis, systolic and diastolic dysfunction, hyperinsulinemia, and impaired myocardial glucose and fatty acid metabolism [77]. Ma *et al.* showed that CYP2J2 overexpression attenuated the high fat diet, streptozotocin-induced diabetic cardiomyopathy in CYP2J2-TG and not WT mice [78]. This was attributed to increased glucose uptake in cardiac and skeletal muscle tissue and lower plasma glucose, triglyceride and insulin levels. CYP2J2-TG showed reduced expression of rate-determining enzymes of the gluconeogenesis pathway such as glucose-6-phosphatase, fructose-1, 6-bisphosphatase and phosphoenolpyruvate carboxykinase. Interestingly, CYP2J2 overexpression prevented thickening of ventricular septum and increase in cardiomyocyte surface area thereby mitigating diabetes-induced cardiac hypertrophy. Additionally, systolic and diastolic dysfunction was partially ameliorated in CYP2J2-TG mice. This was coupled

with upregulation of PPARγ expression, activation of cardiac insulin receptors, AMPK signaling pathways and inhibition of nuclear factor of activated T-cells cytoplasmic 3 (NFATc3) signaling.

Electrophysiologically**,** CYP2J2-TG mice demonstrated enhanced cardiac L-type Ca^{2+} and K_{ATP} currents. It was found out that there was a shortening of cardiac action potential duration due to increased transient outward K^+ (I_{to}) currents [79]. Other outward K^+ currents such as I_{Kr} and I_{Ks} as well as voltage-gated Na^+ currents were comparable between CYP2J2-TG and WT mice. The prolongation of action potential duration is a hallmark of arrhythmogenic changes in heart failure attributed partially to decreased I_{to} currents [80]. Thus overexpression of CYP2J2 may reverse the ion-channel remodeling and prevent the arrhythmogenesis in heart failure.

Abdominal aortic aneurysm (AAA) is a pathological conditions with complex inflammatory signaling pathways contributing to the pathogenesis [81]. AAA is characterized by increase in localized aortic diameters, extensive elastin fragmentation, migration of macrophages, mast cells and natural killer cells to the aortic tissue, and elevation of serum inflammatory cytokines such as interleukins and interferon-γ [81]. It was demonstrated that CYP2J2 overexpression in abdominal aortic tissue in $ApoE^{-/-}$ mice led to lower gene expression and activities of MMP-2 and MMP-9, and inhibition of aortic elastin degradation [82]. CYP2J2 overexpression also led to the reduced expression and serum levels of inflammatory cytokine such as interleukin-6, interleukin-1β, aortic inflammation and macrophage recruitment in vascular smooth muscle cells. Finally, the researchers determined a 3-fold lower incidence of AAA in CYP2J2 overexpressed mice and a 1.5-fold reduction in maximal aortic diameters. [82]. Thus, it was concluded that CYP2J2 overexpression indeed minimized the angiotensin II-induced AAA in $ApoE^{-/-}$ mice.

Since CYP2J2 overexpression has been proven to be highly beneficial in mitigating

adverse cardiac conditions, efforts are being made to overexpress CYP2J2 in other tissues. Tie2-CYP2J2-Tr transgenic mice were generated to produce endothelial cell-specific overexpression of CYP2J2. These mice exhibited mitigation of non-alcoholic fatty liver disease (NAFLD) [83], hepatic hyperlipidemia [84] and adiposity and vascular dysfunction in mice fed with high-fat diet [85].

In all of the above studies, the researchers reported an increase in EET biosynthesis in CYP2J2-TG mice. It is unknown whether metabolism of other PUFAs is increased as well. It has been shown previously that 11,12-EEQ (EPA metabolite) is more potent in activating cardiac K_{ATP} channels than 11,12-EET in murine ventricular cardiomyocytes [86] while 13,14-EDP (DHA metabolite) is 100 times more potent than 11,12-EET in activating BK_{Ca} channels in porcine coronary smooth muscle cells [87]. It is highly likely that the metabolism of other PUFAs is also augmented in CYP2J2-TG mice and the observed cardioprotective effects are possibly due to combinatorial effects of metabolites derived from AA and other PUFAs.

In summary, numerous studies reinforce the importance of CYP2J2 in cardiac homeostasis and the mitigation of adverse cardiac events. With the advent of novel gene editing techniques like CRISPR-Cas9 or hematopoietic-stem-cell gene delivery system, CYP2J2 overexpression in human cardiac tissue holds great promise. Along similar lines, a number of sEH inhibitors have been developed in order to increase the intracellular EETs [88] that mitigate a wide range of cardiovascular disorders [89].

3.3 Implications of CYP2J2 inhibition on cardiac pathophysiology

It is unknown whether CYP2J2 inhibition can indeed lead to cardiac dysfunction although there are apparent correlations between CYP2J2 inhibitors and cardiotoxicity. For example, danazol, a potent CYP2J2 inhibitor, is known to cause congestive heart failure *in vivo* at steady-state plasma concentrations of 0.09-0.5 μM [90]. Drugs that inhibit CYP2J2 have also

been correlated with proarrhythmic effect clinically (Table 3) [91]. For instance, astemizole and terfenadine, second generation anti-histamines were withdrawn from the market due to their significant proarrhythmic effects. Both drugs, inhibit the rapid potassium channel (I_{Kr}) encoded by the human Ether-à-go-go-Related Gene (hERG) causing QT prolongation and the life-threatening *torsades de pointes* tachyarrhythmia [92]. At the same time, astemizole and terfenadine inhibit CYP2J2 potently in primary human cardiomyocytes [48]. Coupled with such CYP2J2 inhibitory activities, it is possible that CYP2J2 inhibition could perhaps contribute to the proarrhythmic effect of astemizole and terfenadine via the inhibition of EET biosynthesis. As the inhibitory potencies of an inhibitor may vary with the probe substrates [93], it becomes important to establish such variability in terms of CYP2J2 inhibition. Until now, CYP2J2 inhibitory potencies have been determined using astemizole and/or terfenadine as probe substrates. As both are xenobiotics, they do not represent the endogenous milieu. Beyond these probe substrates, it has not been proven whether the inhibitors of CYP2J2 also inhibit CYP2J2-mediated AA metabolism. For example, Lee *et al.* reported danazol as a substrate-independent inhibitor of CYP2J2 using albendazole, astemizole, terfenadine and ebastine as probe substrates but not AA [94]. Moreover, other endogenous CYP2J2 substrates such as DHA, EPA and LA must also be tested as probe substrates in CYP2J2 inhibition studies as their metabolites play important roles in cardiophysiology. For instance, the inhibition of DHA metabolism by xenobiotics and the subsequent increase in DHA concentration may inhibit and perturb AA metabolism [24]. Such complex metabolic interactions need to be quantitatively modeled when predicting the cardiotoxic potential of CYP2J2 inhibitors.

Considering the potential cardiac adverse effects due to the inhibition of CYP2J2, the downstream inhibition of sEH and augmentation of cardiac EETs present a novel therapeutic strategy in treating cardiovascular diseases [89]. Nonetheless, it is imperative to thoroughly

evaluate the tumorigenic potential of sEH inhibitors as EETs have been implicated in neoplastic phenotypes *in vitro* [18] and *in vivo* [17].

4. Opportunities in cancer therapy

4.1 Upregulation of CYP2J2 in cancer

Accumulating evidence indicates that CYP2J2 is highly expressed in a variety of human-derived cancer cells and tumors. In a study conducted by Jiang *et al.* using RT-PCR, immunoblotting, and immunohistochemical staining [18], abundant CYP2J2 mRNA and protein were observed in a variety of established human carcinoma cell lines (LS-174, ScaBER, SiHa, U251, A549, Tca-8113, Ncl- H446, and HepG2), whereas CYP2J2 mRNA expression was not detected in non-carcinoma cell lines (HT-1080 and HEK293). In an assortment of human tumor samples, CYP2J2 expression was markedly elevated relative to adjacent non-tumor tissues in 101 of 130 (77%) cancer patients with various types of carcinoma [18]. In a separate study, CYP2J2 was found to be upregulated in 36 out of 42 (86%) patients with hematologic malignant diseases [17]. Collectively, these results suggest that upregulation of CYP2J2 is a general phenomenon in human carcinomas, rather than being tumor type-specific. Given the varied origin of the tumors and cell lines examined, the upregulation of CYP2J2 suggests a common regulatory pathway that may contribute to the neoplastic phenotype of tumor cells. Although CYP2J2 is not induced by commonly studied CYP450 inducers [48], it has been speculated that PPARγ and/or PPARα may regulate CYP2J2 expression as both these nuclear receptors participate primarily in lipid metabolism [95]. On the other hand, it is also unknown whether PPARγ and/or PPARα regulate CYP2J2 in cardiovascular tissues as well.

4.2 Functions of CYP2J2 in cancer

To clarify the contribution of CYP2J2 to the neoplastic phenotype of carcinoma cells, Jiang *et al*. [18] conducted a series of experiments. Forced overexpression of CYP2J2 in cultured carcinoma cell lines (Tca-8113, A549, Ncl-H446,and HepG2) *in vitro* markedly accelerated proliferation and protected them from apoptosis induced by TNFα in cultures, whereas transfection of the antisense recombinant adeno-associated viral CYP2J2 vector (rAAV-antiCYP2J2) or administration of the epoxygenase inhibitor, 17-octadeynoic acid (17-ODYA), inhibited proliferation and accelerated cell apoptosis. Moreover, administration of exogenous EETs mimicked the effects of CYP2J2 overexpression, further implying the production of EETs by CYP2J2 might be responsible for the observed effects. Using Tca-8113 cells as a representative carcinoma cell line, the authors discovered that CYP2J2 and EETs stimulated cell proliferation *in vitro* through phosphorylation of epithelial growth factor receptor (EGFR), and activation of downstream PI3k-AKT and MAPK signaling pathways. Using an *in vivo* murine xenograft model of tumor formation, carcinoma cells overexpressing CYP2J2 generated tumors at a faster rate with increased size compared to those generated from control carcinoma cells. In contrast, tumor formation and size were blunted by antiCYP2J2 transfection in mice. Furthermore, two of the mice injected with rAAV-antiCYP2J2-infected cells did not develop any detectable tumors. In addition to inducing tumor cell proliferation, Jiang *et al*. discovered that CYP2J2 overexpression or EET supplement promotes tumor metastasis [96]. In four different human cancer cell lines (Tca-8113, A549, Hcl-H446, and HepG2), overexpression of CYP2J2 or addition of synthetic EETs significantly induced transwell migration (4.5- to 5.5-fold), invasion of cells (3- to 3.5-fold), cell adhesion to fibronectin, and colony formation in soft agar. In contrast, administration of 17-ODYA or infection with rAAV-antiCYP2J2 inhibited cell migration, invasion, and adhesion with an associated reduction in EETs production. In addition, CYP2J2 overexpression also enhanced metastatic potential *in vivo*. rAAV-CYP2J2-infected MDA-MB- 231 human breast carcinoma

cells showed 60% more lung metastases in athymic BALB/c mice and enhanced angiogenesis in and around primary tumors compared with control cells, whereas lung metastasis was abolished by infection with rAAV-antiCYP2J2. Moreover, CYP2J2 overexpression or EET treatment influences expression of metastasis-related genes in MDA-MB-231 cells and in excised tumor xenografts, including the upregulation of the prometastatic MMP proteins and CD44, and downregulation of antimetastatic genes CD82 and nm23. Conversely, treatment with 17-ODYA or infection with rAAV-antiCYP2J2 decreased expression of MMP-9 and CD44 and increased expression of CD82 and nm-23.

Similarly, Chen *et al*. found that increased EET levels due to CYP2J2 overexpression or adding exogenous EETs in cultured human-derived malignant hematologic cell lines (K562, HL-60, and Raji) significantly accelerated cell proliferation and attenuated apoptosis [17]. In addition, the selective CYP2J2 inhibitor, C26 (1-[4-(vinyl) phenyl]-4-[4-(diphenyl-hydroxymethyl)-piperidinyl]-butanone hydrochloride) inhibited cell proliferation and increased apoptosis, an effect that was significantly reversed by adding EETs [97]. These results suggest that CYP2J2 plays a key role in the pathogenesis of human hematologic malignant diseases.

Taken together, CYP2J2 and EETs play crucial roles in the pathogenesis of various human cancers. CYP2J2 may represent a novel biomarker and a promising antitumor therapeutic target. Inhibition of CYP2J2-mediated EET biosynthesis may provide a novel approach for the treatment of human cancers.

4.3 Inhibitors of CYP2J2 with potential antitumor effects

Chemical derivatives of terfenadine have been shown to be selective and high-affinity inhibitors of human CYP2J2 [50]. Based on these inhibitors, Chen *et al*. synthesized a series of novel terfenadine derivatives as hydrochloride salts for ease of administration [97]. These selective inhibitors of CYP2J2 markedly attenuated the neoplastic phenotypes of carcinoma

cells. In Tca-8113 cells, the CYP2J2 inhibitors decreased EETs production by approximately 60%, while not affecting CYP2J2 mRNA or protein expression levels. One of the novel inhibitor C26 inhibited the proliferation of human tumor cells; reduced their adhesion, migration and invasion; and attenuated activation of epithelial growth factor receptor and PI3K/Akt pathways. C26 also activated Caspase-3 and enhanced human tumor cell apoptosis. In addition, C26 treatment attenuated prometastatic signaling and antiapoptotic protein expression in cancer cells. More importantly, in murine xenograft models induced by MDA-MB-435 cells, treatment with C26 significantly repressed tumor growth, decreased metastasis and extends survival time. Since no significant off-target toxicities were observed, C26 holds promise for targeted combination therapy in metastatic diseases.

Tanshinone IIA is one of the major lipophilic constituents of *Salvia miltiorrhiza*, and is known to have anticancer effects against various types of cancer [98]. In a study using human liver microsomes, tanshinone IIA inhibited CYP2J2-mediated astemizole *O*-demethylation activity dose-dependently and non-competitively [47]. It significantly decreased the viability of human hepatoma HepG2 cells and SiHa cervical cancer cells, whereas it showed no cytotoxicity effects against mouse hepatocytes. Since the mouse hepatocytes have low expression level of CYP2J2, the author hypothesized the species differential effects of tanshinone IIA might be associated with differential CYP2J2 expression. Furthermore, treatment of cells with tanshinone IIA significantly increased the apoptotic cell death rate, as shown by the increase in Annexin V-stained cell populations, Bcl-2 associated X protein (Bax)/B-cell lymphoma 2 (Bcl-2) ratio, and poly (ADP-ribose) polymerase 1 (PARP-1) cleavage in HepG2 cells. Therefore, apoptotic cell death might be a major mechanism of cytotoxicity induced by tanshinone IIA. Additionally, tanshinone IIA treatment significantly decreased HepG2 cell-based tumor growth in nude mice in a dose-dependent manner. However, the tanshinone IIA-induced apoptotic cell death rate was markedly attenuated by

upregulation of CYP2J2 expressionthus exerting its anticancer effects via inhibition of CYP2J2.

Similar to tanshinone IIA, decursin is another naturally derived compound from *Angelica gigas N.* that has shown significant anti-tumor activities [46]. Lee *et al.* reported the inhibition of CYP2J2 activity by decursin and its significant anti-proliferative effects in HepG2 cells. Moreover, similar cytotoxic effects were not observed in primary mouse hepatocytes suggesting the cancer selective effect of decursin. Table 4 summarizes the CYP2J2 inhibitory and anti-proliferative potencies of several known CYP2J2 inhibitors.

Recent advances in cancer research highlight the role of microRNAs (miRNAs) in cancer prognosis and therapy. As the biogenesis and regulation of miRNAs is disrupted in cancer, they may serve as early diagnosis biomarkers [99]. It was shown that let-7b expression was significantly reduced in cancer cell lines such as HeLa, Tca-8113, MDA-MB-435 and SK-MES-1 while CYP2J2 expression was significantly upregulated in these cell lines [100]. In the same study, it was shown that let-7b reduced cancer cell growth and metastasis, induced apoptosis by directly downregulating CYP2J2 expression. This study emphasizes the potential therapeutic roles of miRNAs in cancer therapy and opens avenues for the design of novel anti-CYP2J2 drugs.

As cyclophosphamide and a number of tyrosine kinase inhibitors (TKIs) undergo metabolism by CYP2J2, their intratumoral exposure levels may be influenced by CYP2J2 overexpression [42,101]. From a pharmacokinetic perspective, it is postulated that CYP2J2 inhibition could be a novel strategy to increase intratumoral exposure of such anticancer drugs. Hence, CYP2J2 inhibitors (e.g. amiodarone, danazol and telmisartan) are being considered as adjuvant anticancer therapy along with TKIs. Taken together, CYP2J2 inhibitors are potential anticancer drugs that act either via direct pharmacodynamic or indirect pharmacokinetic mechanism.

One consideration in developing CYP2J2 inhibitors as anticancer drugs is its potential to cause cardiac adverse effects via inhibition of AA metabolism and subsequent reduction of cardiac EETs. As a recap, EETs perform myriad cardioprotective functions such as vasodilation, anti-inflammation, anti-apoptosis and angiogenesis and thus a decrease in intracardiac EETs may trigger or contribute to cardiac adverse effects. Currently, novel CYP2J2 inhibitors developed for anticancer therapy such as C26 are not being studied for their potential to cause drug-induced cardiotoxicity. To realize the full potential of CYP2J2 inhibitors in cancer therapy, it becomes imperative to systematically investigate their cardiac safety profiles.

5. Conclusion and future directions

This commentary discusses the roles of CYP2J2 in cardiac pathophysiology and the opportunities of CYP2J2 inhibition in cancer therapy. In this conclusion, we summarize the current gaps and future directions in CYP2J2 research. Firstly, for drugs that are distributed widely to the heart tissue, their metabolic clearance by cardiac CYP2J2 needs to be investigated in future pharmacokinetic studies. Secondly, while homology model of CYP2J2 has been constructed, the generation of the X-ray crystal structure of CYP2J2 remains important for establishing its structural-activity relationships with both its substrates and inhibitors. Thirdly, while the metabolic outcomes of CYP2J2 overexpression on the metabolism AA has been established, future work is needed to establish the metabolic consequences related to other PUFAs such as DHA, EPA and LA. This is essential to correlate the metabolic perturbations more comprehensively and accurately with the biological endpoints. Fourthly, while the downstream inhibition of sEH and augmentation of cardiac EETs present a novel therapeutic paradigm in cardiovascular diseases, it is imperative to evaluate thoroughly the tumorigenic potential of sEH inhibitors as EETs have been implicated

in neoplasia. Fifthly, as the transcription regulation of CYP2J2 is unknown in both heart and cancer tissues, future work is needed to clarify this knowledge gap. Last but not least, while the inhibition of CYP2J2 presents itself as an attractive anticancer paradigm, future cardiac safety assessment of CYP2J2 inhibitors remains critical to ensure both the efficacy and safety of the new therapy.

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Table 1:

Regioisomeric metabolites deriving from human CYP2J2 metabolism of arachidonic acid, linoleic acid, eicosapentaenoic acid and docosahexaenoic acid.

(Substrate) Regioisomeric Metabolites	% of total	% of R,S	% of S, R	References
(Arachidonic acid)				
5, 6-EET	4	n.d.	n.d.	[13]
8, 9-EET	28	60	40	[13]
11, 12-EET	27	62	38	[13]
14, 15-EET	41	43	57	[13]
(Linoleic acid)				
12,13-EOA	59	n.d.	n.d.	[14]
9,10-EOA	41	n.d.	n.d.	[14]
(Eicosapentaenoic acid)				
5,6-EEQ	3	n.d.	n.d.	[22]
8,9-EEQ	15	n.d.	n.d.	[22]
11,12-EEQ	15	n.d.	n.d.	[22]
14,15-EEQ	16	n.d.	n.d.	[22]
17,18-EEQ	50	65	35	[22]
(Docosahexaenoic acid)				
4,5-EDP	n.d.	n.d.	n.d.	[22]
7,8-EDP	7	n.d.	n.d.	[22]
10,11-EDP	8	n.d.	n.d.	[22]
13,14-EDP	8	n.d.	n.d.	[22]
16,17-EDP	n.d.	n.d.	n.d.	[22]
19,20-EDP	77	75	35	[22]

Table 2.

The relative potencies of mechanism-based inactivators of CYP2J2.

Inactivators	K_I (μM)	K_{inact} (min^{-1})	K_{inact}/K_I (min^{-1} μM^{-1})	*r*	References
Compound 5	0.45±0.05	0.08±0.02	0.046	n.d.	[50]
Compound 13	2.9±0.2	0.47±0.05	0.049	18	[50]
Ritonavir	0.03 ± 0.02	0.034 ± 0.0015	1.1	n.d.	[51]
Dronedarone	0.05 ± 0.01 0.034	0.034 ± 0.0013	0.68	3.3	[39]
Amiodarone	0.21 ± 0.1	0.015 ± 0.0019	0.069	20.7	[39]
NDBD	0.48 ± 0.07	0.024 ± 0.0008	0.049	21.7	[39]
K_{inact}	maximum inactivation rate constant				
K_I	inactivator concentration at half-maximum inactivation rate constant				
K_{inact}/K_I	Potency of inactivation				
r	Partition ratio				

n.d: not determined

Table 3.

Correlation between CYP2J2 inhibition and proarrhythmic effects of marketed drugs

Drug	Type of proarrhythmic effect [91]	rCYP2J2 inhibition (IC_{50} μM)	Substrate	References
Bepridil	QT prolongation	11.5	Astemizole	[43]
Tamoxifen		9.1	Astemizole	[49]
Ketoconazole		5.4	Astemizole	[16]
Mefloquine		22.4	Terfenadine	[49]
Astemizole		8.4	Terfenadine	[49]
Terfenadine		8.1	Ebastine	
Paroxetine		39	Astemizole	[49]
Sertraline		18.5	Astemizole	[43]
Chlorpromazine		24.4	Astemizole	[43]
Haloperidol		14.7	Astemizole	[43]
Thioridazine		12.9	Astemizole	[49]
Pimozide		6.6	Astemizole	[49]
Cisapride		3.2	Terfenadine	[49]
Paroxetine	Brugada syndrome	39	Astemizole	
Perphenazine		10.6	Astemizole	
Thioridazine		12.9	Astemizole	

Table 4.

Comparison of CYP2J2 inhibitory and antiproliferative activities of novel anticancer drugs

Inhibitor	CYP2J2 inhibition			Antiproliferative activity		References
	(IC_{50}) μM	(K_i) μM	Probe substrate	(IC_{50}) μM	Assay format	
Decursin	6.95 17.1	8.34 15.8	Astemizole Terfenadine	5.48	CCK-8 in HepG2 cells	[46]
Tanshinone IIA	2.5	n.d.	Astemizole	N.A.	CCK-8 in HepG2 cells	[47]
Compound 4	0.4	n.d.	Ebastine	10	Ki67 immunohistochemistry in Tca-8113 cells[1]	[97]
Compound 5[1]	0.4	n.d.	Ebastine	10	Ki67 immunohistochemistry in Tca-8113 cells[2]	[97]
Compound 11	4.2	n.d.	Ebastine	<10	Ki67 immunohistochemistry in Tca-8113 cells[2]	[97]
Compound 26		n.d.		<10	MTT assay in Tca-8113, HeLa, A549, and MDA-MB-435 cells; Ki67 immunohistochemistry in Tca-8113 cells[2]	[97]

n.d: not determined

[1]Compound 5 is also a mechanism-based inactivator of CYP2J2. Refer to Table 2.

[2]Percentage of Ki67 immunopositive Tca-8113 cells on treatment with CYP2J2 inhibitors.

FOOTNOTES

This work was supported by the Singapore Ministry of Education Tier 1 Academic Research Funding [Grant R-148-000-193-112] and the National University of Singapore, Department of Pharmacy, Final Year Project Funding [Grant C-148-000-003-001] provided to Eric Chun Yong Chan.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

BCP-D-17-00116