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Assessment of the potential drug-drug interaction between carvedilol and clopidogrel mediated through intestinal P-glycoprotein

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The most widely prescribed oral antiplatelet agent, clopidogrel, shows high interindividual variability resulting in an increased risk of cardiovascular events in the patients with reduced platelet inhibition. The purpose of this study was to investigate the role of the P-glycoprotein (P-gp) efflux pump in limiting the intestinal permeability of clopidogrel and the effect of a β-blocker, namely, carvedilol, on its intestinal transport. Effective permeabilities (P_{eff}) of clopidogrel and carvedilol were investigated in the proximal jejunum and distal ileum of rats using an *in situ* intestinal perfusion model. P_{eff} values of clopidogrel and carvedilol were found to be concentration dependent with decreased P_{eff} values at the low perfusate concentrations. Coperfusion with the P-gp inhibitors verapamil (100 μM) and carvedilol (10 μM) significantly increased the P_{eff} of clopidogrel in the jejunum ($8.31\pm0.20 \times 10^{-5}$ and $6.98\pm0.75 \times 10^{-5}$ vs. $3.60\pm0.51 \times 10^{-5}$, respectively) and ileum ($9.08\pm2.19 \times 10^{-5}$ and $8.35\pm1.58 \times 10^{-5}$ vs. $3.85\pm0.15 \times 10^{-5}$, respectively). However, at the highest concentration tested (30 μM), clopidogrel exhibited 3 and 1.4 times higher P_{eff} than those of metoprolol, an FDA high permeability reference standard, in the jejunum and ileum, respectively. Overall, this study indicates that the efflux function appears not to have a significant impact on the *in vivo* intestinal absorption of clopidogrel due to the saturation of P-gp, suggesting no clinically relevant interaction between carvedilol and clopidogrel mediated through P-gp at intestinal level.

1. Introduction

Nowadays, clopidogrel (Table) is the most widely prescribed oral antiplatelet agent used either alone or in combination with aspirin as dual therapy in the treatment of atherothrombotic cardiovascular disorders and prevention of stroke (Small et al. 2010; Jiang et al. 2015). It is a prodrug that needs to be converted *in vivo* into its active metabolite in a two step process in which the hepatic *cytochrome P450 (CYP) isoenzymes*, namely, *CYP1A2*, *CYP2B6*, *CYP2C9*, *CYP2C19*, and *CYP3A4*, *5*, and as recently emerged, the *paraoxonase 1* (*PON1*) are involved (Sangkuhl et al. 2010; Bouman et al. 2011). The active metabolite inhibits the *adenosine diphosphate (ADP)* mediated platelet activation and aggregation via the antagonism of *ADP* receptor, $P2Y_{12}$ (Savi and Herbert 2005). However, the platelet response to clopidogrel shows high interindividual variability, and the patients with reduced platelet inhibition have an increased risk for cardiovascular events due to the poor response to clopidogrel (Frelinger et al. 2013). It has been estimated that 20-40% of the population is resistant to clopidogrel (Sangkuhl et al. 2010). On the other hand, bleeding complications are also known (Fu et al. 2016). Alterations in the intestinal absorption and metabolic activation have been suggested as possible determinants of the variability in the response to clopidogrel (Frelinger et al. 2013; Wang et al. 2015b). *CYP2C19* plays a central role in the metabolism of clopidogrel, and genetic polymorphisms in *CYP2C19* have been found to strongly correlate with the increased cardiovascular event rates (Jiang et al. 2015). Most recently, several studies have suggested that the variabilities in *CYP3A4*, 5, *CYP2C9* and the esterase *PON1* activities are also associated with the poor response to clopidogrel (Bouman et al. 2011; Brandt et al. 2007; Park et al. 2013; Hokimoto et al. 2014). However, the effects of *CYP3A4*, *CYP3A5* and *PON1* activities on clopidogrel response are still elusive (Hokimoto et al. 2014; Kreutz et al. 2013; Mega et al. 2016). A significant portion of the variability is complex and due to still unknown factors (Frelinger et al. 2013). The nongenetic factors influencing clopidogrel response include possible drug-drug interactions (DDIs), diet, smoking, ethnicity, gender, age, body mass index, drug compliance and coexisting diseases (Frelinger et al. 2013; Xie et al. 2011). Emerging evidence indicates that proton pump inhibitors (e.g. omeprazole), calcium channel blockers (CCBs) and certain statins attenuate the antiplatelet effect of clopidogrel by inhibiting the hepatic *CYP2C19* and *CYP3A4 isoenzymes* with an increased risk of recurrent myocardial infarction and death (Guérin et al. 2016; Gremmel et al. 2015; Pelliccia et al. 2015).

In addition, clopidogrel has been identified as a substrate of P-glycoprotein (P-gp), a membrane efflux pump associated with multidrug resistance 1 (*MDR1*, *ABCB1*) gene, suggesting that the intestinal absorption is limited by P-gp mediated efflux, and a part of the interindividual variability in clopidogrel response might be explained by *MDR1* genotype related differences in functional P-gp expression (Taubert et al. 2006). However, this issue remains controversial in recent studies considering *ABCB1* genotypes and the clinical outcomes of the patients receiving clopidogrel (Jaitner et al. 2012; Price et al. 2012). Hence, the significance of the fact that clopidogrel is a substrate for the intestinal efflux by P-gp remains to be further investigated. Moreover, comedication of clopidogrel with the drugs that also interfere with P-gp transporter might affect the pharmacokinetics (PKs) and antiplatelet effect of clopidogrel. Carvedilol (Table) is a nonselective β-blocking agent with α1-adrenergic antagonist activity. Compared with other antihypertensive drugs, it has been used as a cardioprotective agent (Leonetti and Egan 2012), and widely co-administered with clopidogrel in patients with chronic heart failure. A previous investigation indicated that P-gp is likely to be one of the determinants of carvedilol disposition in humans (Bachmakov et al. 2006). Moreover, carvedilol was shown to significantly inhibit the P-gp function and contribute to drug interactions such as digoxin-carvedilol and cyclosporine-carvedilol (Aiba et al. 2005; Amioka et al. 2007). However, there are no reports regarding the potential

effect of carvedilol on the PKs and pharmacodynamics (PD) of clopidogrel mediated by P-gp.

The purpose of this study was to investigate the role of P-gp in clopidogrel intestinal permeability and the potential effect of carvedilol on its intestinal transport when both are administered together. The regional dependent intestinal permeability and the role of P-gp efflux pump in modulating the intestinal transport of carvedilol and clopidogrel were investigated by an *in situ* intestinal perfusion technique in rats.

2. Investigations and results

2.1. Regional differences in permeability across the rat small intestine

Effective permeability (P_{eff}) values of carvedilol and clopidogrel obtained following the perfusion of proximal jejunum and distal ileum of rats with buffers containing different initial carvedilol and clopidogrel concentrations are presented in Figs. 1 and 2, respectively. P_{eff} values of carvedilol in the proximal jejunum and distal ileum were in the range of 1.19±0.18-4.05±0.58 x 10^{-5} and 2.45±0.31-9.43±2.45 x 10^{-5} cm/s, respectively, while the corresponding P_{eff} values of clopidogrel were 1.22±0.06-10.7±1.1 x 10^{-5} and 1.38±0.11-11.5±1.4 x 10^{-5} cm/s, respectively. Both carvedilol and clopidogrel were found to be stable in the perfusate fluids, and displayed a concentration dependent permeability with decreased P_{eff} values following the perfusion of low initial concentrations. At the highest concentration tested, carvedilol (10 µM) exhibited similar P_{eff} values in comparison to metoprolol, the US Food and Drug Administration (FDA) high permeability reference standard in the proximal jejunum (4.05±0.58 x 10^{-5} vs. 3.53±0.62 x 10^{-5} cm/s, p = 0.298) and distal ileum (9.43±2.45 x 10^{-5} vs. 8.46±0.97 x 10^{-5} cm/s, p = 0.554), while P_{eff} values of clopidogrel (30 µM) were 3 and 1.4 times higher than those of metoprolol in the proximal jejunum (10.7±1.1 x 10^{-5} vs. 3.53±0.62 x 10^{-5} cm/s) and distal ileum (11.5±1.4 x 10^{-5} vs. 8.46±0.97 x 10^{-5} cm/s), respectively. P_{eff} values of carvedilol in the distal ileum were 1.4-2.3 times higher than those in the proximal jejunum, suggesting a segmental dependent permeability, and thus an intestinal regional dependency for the absorption of carvedilol. The *in situ* P_{eff} values of clopidogrel in the proximal and distal segments of rat intestine were comparable. No significant differences (p > 0.05) were found between P_{eff} values in the jejunum and ileum except for the case following the intestinal perfusion of 10 µM clopidogrel where P_{eff} was 1.6 times higher in the jejunum than the ileum.

2.2. Effect of P-gp inhibition on intestinal permeability

For carvedilol and clopidogrel, comparison of P_{eff} values obtained following the perfusion of carvedilol or clopidogrel alone, together and in the presence of a known P-gp inhibitor, verapamil, in the proximal jejunum and distal ileum are presented in Figs. 3 and 4, respectively. Coperfusion of neither verapamil (100 µM) nor clopidogrel (20 µM) significantly affected the P_{eff} of carvedilol in the jejunum and ileum due to the saturation of intestinal P-gp following the perfusion of 10 µM carvedilol (p = 0.637 and 0.943 for coperfusion with verapamil, and p = 0.467 and 0.362 for coperfusion with clopidogrel in the jejunum and ileum, respectively.) However, verapamil (100 µM) and carvedilol (10 µM) increased the P_{eff} of clopidogrel approximately 2 times in the proximal jejunum (8.31±0.20 x 10^{-5} and 6.98±0.75 x 10^{-5} vs. 3.60±0.51 x 10^{-5}, respectively) and distal ileum (9.08±2.19 x 10^{-5} and 8.35±1.58 x 10^{-5} vs. 3.85±0.15 x 10^{-5}, respectively).

3. Discussion

It is well known that P-gp may play a central role in the PKs of many P-gp substrate drugs, and ultimately their clinical responses (Ieiri 2012; Lin and Yamazaki 2003). P-gp expressed in the intestinal epithelial cells has been shown to affect the intestinal absorption and oral bioavailability (BA) of some P-gp substrate drugs due to intestinal efflux (Varma et al. 2005).

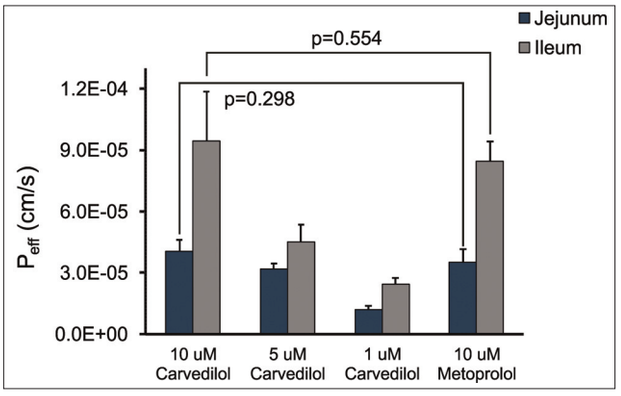


Fig. 1: Effective permeability (P_{eff}, cm/s) values obtained for different perfusate concentrations of carvedilol in comparison to metoprolol (10 µM) following *in situ* intestinal perfusion to the rat proximal jejunum and distal ileum. Data are presented as the mean±SD, n = 3 in each experimental group.

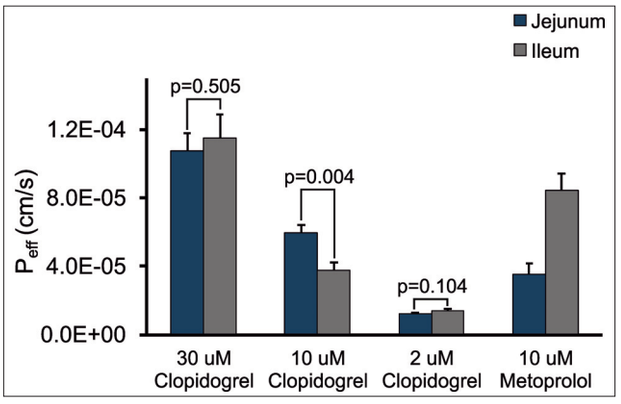


Fig. 2: Effective permeability (P_{eff}, cm/s) values obtained for different perfusate concentrations of clopidogrel in comparison to metoprolol (10 µM) following *in situ* intestinal perfusion to the rat proximal jejunum and distal ileum. Data are presented as the mean±SD, n = 3 in each experimental group.

In the present study, the potential drug-drug interaction (DDI) between clopidogrel and carvedilol via the competition for intestinal P-gp was characterized. The nonselective β-blocker carvedilol is the drug of choice used in coronory heart disease (Rosendorff et al. 2015), while clopidogrel is the most widely prescribed antiplatelet drug worldwide (Topol and Schork 2011). Studies have reported a highly variable antiplatelet response among clopidogrel treated patients (Sangkuhl et al. 2010; Frelinger et al. 2013). This is the first demonstration of regional dependent contribution of P-gp transporter to the intestinal permeability of clopidogrel and carvedilol using *in situ* rat intestinal perfusion. It was previously demonstrated that the rat perfusion model provides a better understanding of the factors that control the intestinal absorption of drugs in humans based on the correlation between the human and rat intestinal permeabilities of drugs (Fagerholm et al. 1996; Cao et al. 2006). Indeed, rat and human show similar transporter expression patterns in the intestine, and there is a reasonable correlation in transporter expression levels between rat and human small intestine (Cao et al. 2006). Therefore, rat permeability data can be used to predict *in vivo* intestinal absorption in humans regardless of the difference in the drug's bioavailability and metabolism in two different species (Cao et al. 2006; Kim et al. 2006; Lennernäs 2007). Studies on rat and human intestine demonstrated that the functional expression of P-gp increases approximately 6 times from proximal to distal regions of the small intestine in both rats and humans (Cao et al. 2005; Englund et al. 2006). It was indicated that the expression of P-gp varies along the intestine, with moderate expression in the duodenum and jejunum, highest

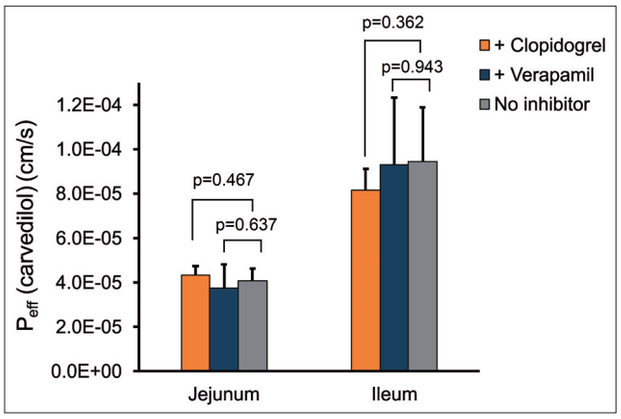


Fig. 3: Effective permeability (P_{eff}, cm/s) values obtained for carvedilol (10 µM) following *in situ* intestinal perfusion to the rat proximal jejunum and distal ileum. Experimental groups include (left to right) a. 20 µM clopidogrel b. 100 µM verapamil c. No inhibitors. Data are presented as the mean±SD, n = 4 in each experimental group.

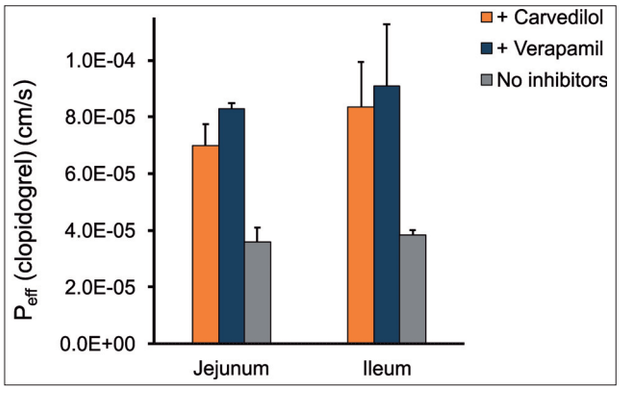


Fig. 4: Effective permeability (P_{eff}, cm/s) values obtained for clopidogrel (20 µM) following *in situ* intestinal perfusion to the rat proximal jejunum and distal ileum. Experimental groups include (left to right) a. 10 µM carvedilol b. 100 µM verapamil c. No inhibitors. Data are presented as the mean±SD, n = 4 in each experimental group.

expression in the ileum, and then a decrease in expression through the colon (Englund et al. 2006; Tamura et al. 2003). However, discrepancies in P-gp expression between human and rat intestine were also reported. The comparison of the expression levels of the *MDR1* transporter between humans and rats revealed that the relative expression level of P-gp in human duodenum was considerably higher than that in the rat duodenum, while its expression in the human colon was equal to one half the expression level in the rat colon (Cao et al. 2006; Kim et al. 2007). Therefore, the lower expression of P-gp in the proximal regions of rat intestine may lead to a lesser role for this transporter in the absorption of P-gp substrates from the proximal segments in the rat model compared to human (Dahan et al. 2009a). However, this experimental model in rats has been widely used to investigate the P-gp efflux involved in the transepithelial transport of drugs along the entire small intestine based on the similarity in P-gp expression patterns and the membrane structure similarity between human and rat intestine (Cao et al. 2005; Dahan et al. 2009b, c). Thus, the present perfusion study clarified the impact of P-gp efflux on the intestinal transport of these two drugs. Both carvedilol and clopidogrel exhibited significant concentration dependent increases in permeability values in rat intestine. Higher concentrations of clopidogrel and carvedilol resulted in higher intestinal permeability, consistent with the saturation of the efflux transporter. P-gp inhibition by either carvedilol or verapamil had a significant effect on the intestinal permeability of clopidogrel with a significant increase in its jejunal and ileal P_{eff} values. The inhibitory effect of carvedilol on the P-gp drug efflux pump has been demonstrated before by the other investigators where a concomitant use of carvedilol decreased the oral clearance of digoxin by half and resulted in digoxin toxicity in pediatric patients (Ratnapalan et al. 2003). In another study, a single oral dose of 25 mg carvedilol significantly increased the plasma concentrations of orally administered digoxin in healthy adults (De Mey et al. 1990). Moreover, *MDR1* mediated reversing effect of carvedilol was shown to be similar to that of the specific P-gp inhibitor, verapamil, indicating that carvedilol is a candidate modulator of *MDR1* in clinical use and may alter the PKs of drugs and cause P-gp mediated DDIs (Amioka et al. 2007; Kakumoto et al. 2003). The present study also demonstrated that the inhibitory effect of carvedilol was comparable with that of verapamil.

Previously, the uptake and efflux experimental data in Caco-2 cells consistently demonstrated that the efflux clearance of clopidogrel is driven by the efflux transporter, P-gp (Taubert et al. 2006). Taubert et al. provided evidence that P-gp is a key factor for the intestinal absorption of clopidogrel and *MDR1 C3435T* genotype is a potential predictor of clopidogrel PKs. The clinical PK study conducted in patients undergoing percutaneous coronary intervention (PCI) revealed that following the administration of a single clopidogrel dose (300 or 600 mg), the peak plasma concentration (C_{max}) and area under the plasma concentration-time curve (AUC) values of clopidogrel and its active metabolite were significantly lower in patients with the *3435T/T* genotype compared with the carriers of *3435C/T* and *3435C/C* as a result of enhanced intestinal efflux due to a higher P-gp expression associated with the *3435T/T* genotype (Taubert et al. 2006). The result was also supported by other studies where *C3435T* polymorphism of the *MDR1* gene significantly affected the PKs of clopidogrel, its inactive and active metabolites, as well as the clinical outcomes in patients with an acute coronary syndrome (Wang et al. 2015b; Mega et al. 2010). However, it is evident that there are some contradictory results regarding the genetic variations of P-gp involved in clopidogrel therapy (Frelinger et al. 2013; Jaitner et al. 2012; Price et al. 2012; Braun et al. 2013; Karaźniewicz-Łada et al. 2014). In a rigorously controlled study, no associations were observed between the *MDR1 C3435T* polymorphism and clopidogrel PK or PD endpoints (Frelinger et al. 2013). In another study, the generation of active metabolite or on-treatment platelet reactivity (OPR) was found not to be affected by *MDR1* genotype (Braun et al. 2013). Whereas, a recent study demonstrated that the presence of *C3435T* polymorphism had an impact on clopidogrel PKs, but not on its PDs (Karaźniewicz-Łada et al. 2014). These conflicting findings on the impact of *MDR1 C3435T* mutation on cardiovascular outcomes were evaluated in two meta-analyses (Su et al. 2012; Luo et al. 2012). However, the association between the *ABCB1 C3435T* polymorphism and platelet activity and the other risk of poor clinical outcomes were found not to be significant (Su et al. 2012; Luo et al. 2012). Additionally, other efflux membrane transporters such as multidrug resistance associated protein 2 and the breast cancer resistance protein were shown not to interfere in the efflux mechanism of clopidogrel (Taubert et al. 2006).

Consistent with the previous report (Taubert et al. 2006), the present study clearly indicated that P-gp mediated efflux is involved in the intestinal epithelium transport of clopidogrel, and the inhibition by carvedilol had a significant effect on the P_{eff} of clopidogrel following the perfusion of low clopidogrel concentration in the rat model. However, the efflux effect may not always be clinically or practically significant in the outcome (Ogihara et al. 2006; Custodio et al. 2008). Cao et al. (2005) showed that P-gp plays a minimal role in the intestinal absorption of the high permeability P-gp substrate drugs like verapamil. In the present study, the perfusate concentration of clopidogrel was in the range of 2-30 µM which was extremely below the hypothetical gastrointestinal (GI) concentrations (932 and 3,728 µM) based on the daily dose (75 mg) and the highest dose strength (HDS) (300 mg) of clopidogrel dissolved in 250 mL water, the acceptable gastric volume following oral drug ingestion. Thus, P-gp efflux function appears not to have a significant impact on the rate and extent of *in vivo*

intestinal absorption of clopidogrel at therapeutic doses which represent high GI concentration, and thereby the saturation of the efflux pump. In this study, the intestinal efflux was found to be saturated at the highest clopidogrel concentration tested (30 µM) in the perfusion solution. Moreover, the *in situ* P_{eff} of clopidogrel was found to be higher than that of metoprolol, an FDA high permeability reference standard. The same case was also evident for carvedilol where the drug concentration in the perfusion solution (1-10 µM) was extremely below the hypothetical drug concentration (246 µM) based on HDS (25 mg) of carvedilol. P_{eff} of carvedilol was found to be similar to that of metoprolol in both jejunum and ileum following the perfusion of intestine with the highest initial drug concentration (10 µM). Therefore, P-gp inhibition appears not to be clinically significant in the concomitant use of clopidogrel and carvedilol at therapeutic doses due to saturation of the efflux mechanism at the intestinal level. However, the efflux effect may be present at the lower intestinal concentrations of clopidogrel, and may be compensated by the concomitant use of carvedilol, a P-gp inhibitor resulting in a beneficial effect in the treatment. In addition, very little is known regarding the P-gp mediated DDIs of clopidogrel and its contribution to the variability in the intestinal absorption of clopidogrel in humans (Wang et al. 2015a). Harmsze et al. (2010) investigated the effect of co-administration of P-gp inhibiting CCBs (verapamil, nifedipine, diltiazem, barnidipine) or non-P-gp inhibiting CCB (amlodipine) on the OPR of clopidogrel in patients on dual antiplatelet therapy after elective PCI. Both P-gp and non-P-gp inhibiting CCBs increase OPR due to the known *CYP3A4* inhibition by CCBs. However, only amlodipine was associated with the poor response to clopidogrel (Harmsze et al. 2010).

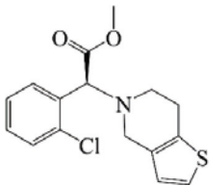
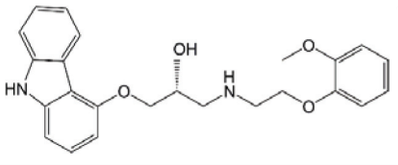
Previous studies on clopidogrel clearly demonstrated that common and functional polymorphisms in *CYP isoenzymes* are mainly responsible for clopidogrel PKs and responsiveness (Brandt et al. 2007). *In vitro* enzyme kinetics studies revealed that *CYP1A2* (35.8 %), *CYP2B6* (19.4 %) and *CYP2C19* (44.9 %) contribute to the formation of 2-oxoclopidogrel intermediate metabolite, whereas *CYP2B6* (32.9 %), *CYP2C9* (6.76 %), *CYP2C19* (20.6 %) and *CYP3A4* (39.8 %) contribute to the formation of the active metabolite (Kazui et al. 2010). It is evident that clopidogrel has a great chance of interactions with other drugs sharing the same metabolism pathways. Carvedilol also undergoes substantial oxidative metabolism mediated by *CYP2D6*, with possible contributions by *CYP1A2*, *CYP2C9*, *CYP2E1* and *CYP3A4* (Shihmanter et al. 2014; Oldham and Clarke 1997). Therefore, the question arises whether carvedilol interferes with clopidogrel metabolism at the level of *CYP isoenzymes,* which needs to be investigated in future studies.

The present study demonstrated for the first time that carvedilol exhibited regional dependent permeability in rats, whereas clopidogrel did not. Indeed, the *in vivo* intestinal absorption of carvedilol and clopidogrel are likely dominated by their high permeability properties leading to the saturation of intestinal P-gp.

In general, physicochemical properties of drugs are the major determinants of the regional intestinal permeability. Clopidogrel and carvedilol are Biopharmaceutics Classification System (BCS) Class 2 (low solubility-high permeability) weak bases (Lassoued et al. 2011; Tsume et al. 2014) (Table). It is evident that the key determinant in the intestinal absorption of BCS Class 2 drugs is the drug solubility in the absorption region of the intestine (Yu et al. 2002). Therefore, the solubilization which can be affected by the GI physiological factors such as pH and surfactants may contribute to the interindividual variability in clopidogrel absorption. Having ionizable groups, carvedilol and clopidogrel demonstrate a pH dependent solubility with decreased solubility at high pHs. Carvedilol (pK_a: 7.8, log P: 4.19) (Loftsson et al. 2008; Benet et al. 2011) and clopidogrel (pK_a: 4.56, log P: 3.9) (Dimopoulou et al. 2015) are completely ionized at pH 1.2. The unionized fractions (f_u) of carvedilol and clopidogrel are nearly zero up to pH 5.5 and pH 2, respectively, and then gradually increase as pH increases giving the sigmoidal profiles around pH 10 and pH 6.5, respectively. The f_u values of clopidogrel in the jejunum (pH 6.0) and ileum (pH 7.4) are high and similar which may explain its high P_{eff} with no regional dependency. Therefore, the dissolution of carvedilol and clopidogrel are likely to be complete in the stomach (pH 1.4-2.1 (Yu et al. 2002)), and entering the upper intestine (pH 4.9-6.4 under fasting conditions (Yu et al. 2002)), they are likely to be present in a supersaturated state, and rapidly absorbed before precipitating at the higher pH values of the small intestine. It is also in agreement with the PK data indicating that carvedilol and clopidogrel are rapidly absorbed after oral administration (Neugebauer et al. 1987; Hurbin et al. 2012). Carvedilol's fraction of dose absorbed in humans was reported to be 65% with the absolute BA of 24% due to the high first-pass effect (Neugebauer et al. 1987; Varma et al. 2012), while the absorption of clopidogrel is at least 50% based on the urinary excretion of clopidogrel metabolites (Hurbin et al. 2012). However, the significant degree of metabolism contributes to the low systemic exposure of intermediate and active metabolites of clopidogrel following oral administration. Upon absorption, hepatic carboxylesterase 1 (CE1) rapidly degrades approximately 85% of absorbed clopidogrel to an inactive carboxylic acid derivative (Huttunen et al. 2011). *CYP isoenzymes* metabolize the remaining (15%) to produce 2-oxoclopidogrel. Half of the intermediate metabolite is further metabolized to the pharmacologically active metabolite (Sangkuhl et al. 2010; Qiu et al. 2014). Furthermore, the cascading active metabolite is partly subjected to deactivation by CE1 in the liver (Qiu et al. 2014).

Overall, the present study demonstrated that high membrane permeability of clopidogrel saturates the P-gp efflux pump along the intestine, subsequently resulting in good oral absorption. Hence, DDI between carvedilol and clopidogrel mediated through P-gp seems not to dominate the intestinal absorption process due to the saturation of efflux function at the level of intestine. On the other hand, the possibility of P-gp interaction cannot be completely ruled out for clopidogrel. It is still possible that the inhibition by carvediol may be a determinant for the effectiveness of clopidogrel therapy, because P-gp is constitutively expressed in the liver and other organs as well. The significance of P-gp mediated efflux in regard to the disposition of clopidogrel and the potential metabolic interaction between carvedilol and clopidogrel need to be further investigated to find out additional explanations to the observed interindividual high variability in the efficacy of clopidogrel.

Table: Chemical structures and physicochemical properties of clopidogrel and carvedilol

Compound	Chemical Structure	pK_a	log P
Clopidogrel		4.56[a]	3.9[a]
Carvedilol		7.8[b]	4.19[c]

[a]Dimopoulou et al. (2015); [b]Loftsson et al. (2008); [c]Benet et al. (2011).

4. Experimental

4.1. Materials

Carvedilol, clopidogrel bisulfate, metoprolol tartrate and verapamil hydrochloride were kindly supplied from Drogsan Pharmaceuticals (Ankara, Turkey), Deva Holding (Istanbul, Tukey), Novartis Pharma AG (Basel, Switzerland) and Abbott Laboratories (Istanbul, Turkey), respectively. Phenol red, calcium chloride, magnesium chloride, sodium dihydrogen phosphate, D-glucose, 2-morpholinoethanesulfonic acid (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), potassium chloride, sodium chloride and HPLC grade acetonitrile were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals were of analytical grade.

4.2. Methods

4.2.1. In situ intestinal perfusion in rats

P_{eff} was investigated using *in situ* intestinal perfusion in the proximal jejunum and distal ileum of rats. The study protocol of animal experiments was approved by the Institutional Ethics Committee of Gazi University, Ankara, Turkey (G.U.ET-13.053). The animals were housed and handled according to the guidelines of Gazi University for Laboratory Animal Experimentation. Male Wistar albino rats (250-300 g) from Kobay Research Animal Production Laboratories (Ankara, Turkey) were used for the perfusion studies. Prior to each experiment, the rats fasted overnight (12-18 h) with free access to water. Animals were randomly assigned to the different experimental groups. The procedure was performed according to the previously published report (Incecayir et al. 2013). Briefly, rats were anesthetized with an im injection of 5 mg/kg xylazine and 80 mg/kg ketamine, and placed on a heated surface maintained at 37 °C. The abdomen was opened by a midline incision of 3-4 cm. The proximal jejunal segment (3 cm average distance of the inlet from the ligament of Treitz) or the distal ileal segment (3 cm average distance of the outlet from the ligament of cecum) of approximately 10 cm was carefully exposed and cannulated on two ends with flexible PVC tubing (Tygon® LFL, 2.29 mm i.d., Harvard Apparatus, Holliston, MA, US). Disturbance of the circulatory system was avoided. The exposed segment was kept moist with normal saline solution at 37 °C. All solutions were kept in a 37 °C water bath. The isolated segment was rinsed with normal saline solution to clean out any residual debris. Perfusion buffers containing 5 mM MES, pH 6.0 and 5 mM HEPES, pH 7.4 were used for the corresponding segments, jejunum and ileum, respectively. Perfusion buffers were prepared with 1 mM calcium chloride, 0.5 mM magnesium chloride, 145 mM sodium chloride, 1 mM sodium dihydrogen phosphate, 3 mM potasium chloride and 5 mM d-glucose. Drug concentrations in the perfusion solution were in the ranges of 1-10 and 2-30 µM for carvedilol and clopidogrel, respectively. Carvedilol (10 µM) was tested in the presence of verapamil (100 µM), a selective inhibitor of P-gp and clopidogrel (20 µM) separately. Clopidogrel (20 µM) was tested in the presence of verapamil (100 µM) and carvedilol (10 µM) in the separate intestinal perfusion studies. Phenol red (0.5 mM) was added to the perfusion buffer as a nonabsorbable marker for water flux measurement. Metoprolol was used as a reference standard for permeability in close proximity to the low/high permeability class boundry in a separate intestinal perfusion in rats. The perfusion buffers containing drug(s) and phenol red were perfused through the intestinal segments by a peristaltic infusion pump (Ecoline 8-Channel, Harvard Apparatus, Holliston, MA, US) at a flow rate of 0.2 mL/min. After an 1 h perfusion to reach steady state, perfusion samples were taken in 10 min intervals for 1 h. Drug concentrations in perfusion samples were analyzed by an ultra performance liquid chromatography (UPLC) method. The length of each perfused intestinal segment was accurately measured at the end of the experiment.

4.2.2. Data analysis

The net water flux in the *in situ* intestinal perfusion studies was determined by the quantification of phenol red, a nonabsorbed and nonmetabolized marker. The C_{out}/C_{in} ratio was corrected for water transport according to Eq. (1):

$$\frac{C'_{out}}{C'_{in}} = \frac{C_{out}}{C_{in}} \cdot \frac{C_{in\ phenol\ red}}{C_{out\ phenol\ red}} \qquad (1)$$

where $C_{in\ phenol\ red}$ and $C_{out\ phenol\ red}$ are equal to the concentrations of phenol red in the inlet and outlet samples, respectively.

P_{eff} (cm/s) through the rat gut wall was determined using the "plug flow" model expressed in Eq. (2) (Fagerholm et al. 1996):

$$P_{eff}\,(cm/s) = \frac{-Q \ln\left(C'out / C'in\right)}{2\pi R L} \qquad (2)$$

where Q is the perfusion buffer flow rate (0.2 mL/min), C'_{out}/C'_{in} is the ratio of the outlet and inlet concentrations of the tested drug that have been adjusted for water transport, R and L are the radius (0.2 cm) and length of the intestinal segment, respectively.

4.2.3. Analytical method

The simultaneous analysis of carvedilol, clopidogrel and verapamil in perfusion samples with phenol red was performed using an UPLC system (An Acquity™ UPLC System, Waters®, Milford, MA, US) with a photodiode array UV detector (Waters®, Milford, MA, US) controlled by Mass Lynx V 4.1 Software. Separation was performed on Acquity™ $BEHC_{18}$ column, 100 mm x 2.1 mm, 1.7 µm (Waters®, Ireland). Mobile phase A contained 0.1% phosphoric acid in water and mobile phase B contained acetonitrile with the mobile phase B gradient changing from 2 to 98% over 2.5 min. The flow rate was 0.25 mL/min at room temperature. Injection volume was 10 µL. The eluate was analyzed at a wavelength of 241 nm. Retention times were 1.6, 2.0, 1.7 and 1.5 min for carvedilol, clopidogrel, verapamil and phenol red, respectively. For simultaneous analysis of metoprolol and phenol red, mobile phase A contained 0.1% phosphoric acid in water and mobile phase B contained 0.1% phosphoric acid in acetonitrile with the mobile phase B gradient changing from 2 to 60% over 10 min. The detection wavelength of metoprolol was 275 nm. Retention times were 4.9 and 5.4 min for metoprolol and phenol red, respectively. Standard curves generated for each drug were utilized for quantitation of integrated area under peaks. Calibration curves were linear over the range of 0.35-50 µM. The within-day and between-day precisions expressed as coefficient of variation were in the range of 0.6 to 2.2%.

4.2.4. Statistical analysis

Perfusion experiments were performed in either triplicate or quadruplicate. No animals or data points were excluded from the reported results. The data are presented as mean±standard deviation (SD). The independent t test was used to assess differences between two groups. Differences were considered statistically significant at $p < 0.05$.

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References

Aiba T, Ishida K, Yoshinaga M, Okuno M, Hashimoto Y (2005) Pharmacokinetic characterization of transcellular transport and drug interaction of digoxin in Caco-2 cell monolayers. Biol Pharm Bull 28: 114-119.

Amioka K, Kuzuya T, Kushihara H, Ejiri M, Nitta A, Nabeshima T (2007) Carvedilol increases ciclosporin bioavailability by inhibiting P-glycoprotein-mediated transport. J Pharm Pharmacol 59: 1383-1387.

Bachmakov I, Werner U, Endress B, Auge D, Fromm MF (2006) Characterization of β-adrenoceptor antagonists as substrates and inhibitors of the drug transporter P-glycoprotein. Fundam Clin Pharmacol 20: 273-282.

Benet LZ, Broccatelli F, Oprea TI (2011) BDDCS applied to over 900 drugs. AAPS J 13: 519-547.

Bouman HJ, Schömig E, van Werkum JW, Velder J, Hackeng CM, Hirschhäuser C, Waldmann C, Schmalz HG, ten Berg JM, Taubert D (2011) Paraoxonase-1 is a major determinant of clopidogrel efficacy. Nat Med 17: 110-116.

Brandt JT, Close SL, Iturria SJ, Payne CD, Farid NA, Ernest CS 2nd, Lachno DR, Salazar D, Winters KJ (2007) Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost 5: 2429-2436.

Braun OÖ, Angiolillo DJ, Ferreiro JL, Jakubowski JA, Winters KJ, Effron MB, Duvvuru S, Costigan TM, Sundseth S, Walker JR, Saucedo JF, Kleiman NS, Varenhorst C (2013) Enhanced active metabolite generation and platelet inhibition with prasugrel compared to clopidogrel regardless of genotype in thienopyridine metabolic pathways. Thromb Haemost 110: 1223-1231.

Cao X, Yu LX, Barbaciru C, Landowski CP, Shin H-C, Gibbs S, Miller HA, Amidon GL, Sun D (2005) Permeability dominates in vivo intestinal absorption of P-gp substrate with high solubility and high permeability. Mol Pharm 2: 329-340.

Cao X, Gibbs ST, Fang L, Miller HA, Landowski CP, Shin H-C, Lennernas H, Zhong Y, Amidon GL, Yu LX, Sun D (2006) Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model. Pharm Res 23: 1675-1686.

Custodio JM, Wu C-Y, Benet LZ (2008) Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. Adv Drug Deliv Rev 60: 717-733.

Dahan A, Amidon GL (2009a) Segmental dependent transport of low permeability compounds along the small intestine due to P-glycoprotein: the role of efflux transport in the oral absorption of BCS class III drugs. Mol Pharm 6: 19-28.

Dahan A, Sabit H, Amidon GL (2009b) Multiple efflux pumps are involved in the transepithelial transport of colchicine: combined effect of P-glycoprotein and multidrug resistance-associated protein 2 leads to decreased intestinal absorption throughout the entire small intestine. Drug Metab Dispos 37: 2028-2036.

Dahan A, Amidon GL (2009c) Grapefruit juice and its constituents augment colchicine intestinal absorption: potential hazardous interaction and the role of P-glycoprotein. Pharm Res 26: 883-892.

De Mey C, Brendel E, Enterling D (1990) Carvedilol increases the systemic bioavailability of oral digoxin. Br J Clin Pharmacol 29: 486-490.

Dimopoulou M, Mourouti C-S, Vertzoni M, Symillides M, Reppas C (2015) In-vitro evaluation of performance of solid immediate release dosage forms of weak bases in upper gastrointestinal lumen: experience with miconazole and clopidogrel salts. J Pharm Pharmacol, doi:10.1111/jphp.12406

Englund G, Rorsman F, Rönnblom A, Karlbom U, Lazorova L, Gråsjö J, Kindmark A, Artursson P (2006) Regional levels of drug transporters along the human intestinal tract: co-expression of ABC and SLC transporters and comparison with Caco-2 cells. Eur J Pharm Sci 29: 269-277.

Fagerholm U, Johansson M, Lennernäs H (1996) Comparison between permeability coefficients in rat and human jejunum. Pharm Res 13: 1336-1342.

Frelinger AL 3rd, Bhatt DL, Lee RD, Mulford DJ, Wu J, Nudurupati S, Nigam A, Lampa M, Brooks JK, Barnard MR, Michelson AD (2013) Clopidogrel pharmacokinetics and pharmacodynamics vary widely despite exclusion or control of polymorphisms (CYP2C19, ABCB1, PON1), noncompliance, diet, smoking, co-medications (including proton pump inhibitors), and pre-existent variability in platelet function. J Am Coll Cardiol 61: 872-879.

Fu A, Singh K, Abunassar J, Malhotra N, Le May M, Labinaz M, Glover C, Marquis J-F, Froeschl M, Dick A, Hibbert B, Chong A-Y, So DYF (2016) Ticagrelor in triple antithrombotic therapy: predictors of ischemic and bleeding complications. Clin Cardiol 39: 19-23.

Gremmel T, Durstberger M, Eichelberger B, Koppensteiner R, Panzer S (2015) Calcium-channel blockers attenuate the antiplatelet effect of clopidogrel. Cardiovasc Ther 33: 264-269.

Guérin A, Mody R, Carter V, Ayas C, Patel H, Lasch K, Wu E (2016) Changes in practice patterns of clopidogrel in combination with proton pump inhibitors after an FDA safety communication. PLoS One 11, e0145504, doi:10.1371/journal.pone.0145504.

Harmsze AM, Robijns K, van Werkum JW, Breet NJ, Hackeng CM, ten Berg JM, Ruven HJT, Klungel OH, de Boer A, Deneer VHM (2010) The use of amlodipine, but not of P-glycoprotein inhibiting calcium channel blockers is associated with clopidogrel poor-response. Thromb Haemost 103: 920-925.

Hokimoto S, Chitose T, Mizobe M, Akasaka T, Arima Y, Kaikita K, Iwashita S, Morita K, Miyazaki H, Oniki K, Matsui K, Nakagawa K, Ogawa H (2014) Impact of CYP3A5 polymorphism on platelet reactivity at percutaneous coronary intervention and after 9 months of aspirin and clopidogrel therapy in Japanese patients with coronary artery disease. Eur J Clin Pharmacol 70: 667-673.

Hurbin F, Boulenc X, Daskalakis N, Farenc C, Taylor T, Bonneau D, Lacreta F, Cheng S, Sultan E (2012) Clopidogrel pharmacodynamics and pharmacokinetics in the fed and fasted state: a randomized crossover study of healthy men. J Clin Pharmacol 52: 1506-1515.

Huttunen KM, Raunio H, Rautio J (2011) Prodrugs-from serendipity to rational design. Pharmacol Rev 63: 750-771.

Ieiri I (2012) Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). Drug Metab Pharmacokinet 27: 85-105.

Incecayir T, Tsume Y, Amidon GL (2013) Comparison of the permeability of metoprolol and labetalol in rat, mouse, and Caco-2 cells: use as a reference standard for BCS classification. Mol Pharm 10: 958-966.

Jaitner J, Morath T, Byrne RA, Braun S, Gebhard D, Bernlochner I, Schulz S, Mehilli J, Schömig A, Koch W, Kastrati A, Sibbing D (2012) No association of ABCB1 C3435T genotype with clopidogrel response or risk of stent thrombosis in patients undergoing coronary stenting. Circ Cardiovasc Interv 5: 82-88.

Jiang X-L, Samant S, Lesko LJ, Schmidt S (2015) Clinical pharmacokinetics and pharmacodynamics of clopidogrel. Clin Pharmacokinet 54: 147-166.

Kakumoto M, Sakaeda T, Takara K, Nakamura T, Kita T, Yagami T, Kobayashi H, Okamura N, Okumura K (2003) Effects of carvedilol on MDR1-mediated multidrug resistance: comparison with verapamil. Cancer Sci 94: 81-86.

Karaźniewicz-Łada M, Danielak D, Rubiś B, Burchardt P, Komosa A, Lesiak M, Główka F (2014) Impact of common ABCB1 polymorphism on pharmacokinetics and pharmacodynamics of clopidogrel and its metabolites. J Clin Pharm Ther 40: 226-231.

Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, Ikeda T, Kurihara A (2010) Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. Drug Metab Dispos 38: 92-99.

Kim H-R, Park S-W, Cho H-J, Chae K-A, Sung J-M, Kim J-S, Landowski CP, Sun D, Abd El-Aty AM, Amidon GL, Shin H-C (2007) Comparative gene expression profiles of intestinal transporters in mice, rats and humans. Pharmacol Res 56: 224-236.

Kim JS, Mitchell S, Kijek P, Tsume Y, Hilfinger J, Amidon GL (2006) The suitability of an in situ perfusion model for permeability determinations: utility for BCS class I biowaiver requests. Mol Pharm 3: 686-694.

Kreutz RP, Owens J, Jin Y, Nystrom P, Desta Z, Kreutz Y, Breall JA, Li L, Chiang C, Kovacs RJ, Flockhart DA (2013) Cytochrome P450 3A4*22, PPAR-α, and ARNT polymorphisms and clopidogrel response. Clin Pharmacol 5: 185-192.

Lassoued MA, Khemiss F, Sfar S (2011) Comparative study of two in vitro methods for assessing drug absorption: sartorius SM 16750 apparatus versus everted gut sac. J Pharm Pharm Sci 14: 117-127.

Lennernäs H (2007) Animal data: the contributions of the Ussing chamber and perfusion systems to predicting human oral drug delivery in vivo. Adv Drug Deliv Rev 59: 1103-1120.

Leonetti G, Egan CG (2012) Use of carvedilol in hypertension: an update. Vasc Health Risk Manag 8: 307-322.

Lin JH, Yamazaki M (2003) Role of P-glycoprotein in pharmacokinetics: clinical implications. Clin Pharmacokinet 42: 59-98.

Loftsson T, Vogensen SB, Desbos C, Jansook P (2008) Carvedilol: solubilization and cyclodextrin complexation: a technical note. AAPS PharmSciTech. 9: 425-430.

Luo M, Li J, Xu X, Sun X, Sheng W (2012) ABCB1 C3435T polymorphism and risk of adverse clinical events in clopidogrel treated patients: a meta-analysis. Thromb Res 129: 754-759.

Mega JL, Close SL, Wiviott SD, Shen L, Walker JR, Simone T, Antman EM, Braunwald E, Sabatine MS (2010) Genetic variants in ABCB1, CYP2C19, and cardiovascular outcomes following treatment with clopidogrel and prasugrel. Lancet 376: 1312-1319.

Mega JL, Close SL, Wiviott SD, Man M, Duvvuru S, Walker JR, Sundseth SS, Collet J-P, Delaney JT, Hulot J-S, Murphy SA, Paré G, Price MJ, Sibbing D, Simon T, Trenk D, Antman EM, Sabatine MS (2016) PON1 Q192R genetic variant and response to clopidogrel and prasugrel: pharmacokinetics, pharmacodynamics, and a meta-analysis of clinical outcomes. J Thromb Thrombolysis 41: 374-383.

Neugebauer G, Akpan W, von Möllendorff E, Neubert P, Reiff K (1987) Pharmacokinetics and disposition of carvedilol in humans. J Cardiovasc Pharmacol 10: S85-S88.

Ogihara T, Kamiya M, Ozawa M, Fujita T, Yamamoto A, Yamashita S, Ohnishi S, Isomura Y (2006) What kinds of substrates show P-glycoprotein-dependent intestinal absorption? Comparison of verapamil with vinblastine. Drug Metab Pharmacokinet 21: 238-244.

Oldham HG, Clarke SE (1997) In vitro identification of the human cytochrome P450 enzymes involved in the metabolism of R(+)- and S(-)-carvedilol. Drug Metab Dispos 25: 970-977.

Park JJ, Park KW, Kang J, Jeon KH, Kang SH, Ahn HS, Han JK, Koh JS, Lee SE, Yang HM, Lee HY, Kang HJ, Koo BK, Oh BH, Park YB, Kim HS (2013) CYP3A4 genetic status may be associated with increased vulnerability to the inhibitory effect of calcium-channel blockers on clopidogrel. Circ J 77: 1289-1296.

Pelliccia F, Rollini F, Marazzi G, Greco C, Gaudio C, Angiolillo DJ (2015) Drug-drug interactions between clopidogrel and novel cardiovascular drugs. Eur J Pharmacol 765: 332-336.

Price MJ, Murray SS, Angiolillo DJ, Lillie E, Smith EN, Tisch RL, Schork NJ, Teirstein PS, Topol EJ (2012) Influence of genetic polymorphisms on the effect of high- and standard-dose clopidogrel after percutaneous coronary intervention: the GIFT (Genotype information and functional testing) study. J Am Coll Cardiol 59: 1928-1937.

Qiu Z, Li N, Song L, Lu Y, Jing J, Parekha HS, Gao W, Tian F, Wang X, Ren S, Chen X (2014) Contributions of intestine and plasma to the presystemic bioconversion of vicagrel, an acetate of clopidogrel. Pharm Res 31: 238-251.

Ratnapalan S, Griffiths K, Costei AM, Benson L, Koren G (2003) Digoxin-carvedilol interactions in children. J Pediatr 142: 572-574.

Rosendorff C, Lackland DT, Allison M, Aronow WS, Black HR, Blumenthal RS, Cannon CP, de Lemos JA, Elliott WJ, Findeiss L, Gersh BJ, Gore JM, Levy D, Long JB, O'Connor CM, O'Gara PT, Ogedegbe O, Oparil S, White WB (2015) Treatment of hypertension in patients with coronary artery disease: a scientific statement from the American Heart Association, American College of Cardiology, and American Society of Hypertension. J Am Soc Hypertens 9: 453-498.

Sangkuhl K, Klein TE, Altman RB (2010) Clopidogrel pathway. Pharmacogenet Genomics 20: 463-465.

Savi P, Herbert JM (2005) Clopidogrel and ticlopidine: P2Y12 adenosine diphosphate-receptor antagonists for the prevention of atherothrombosis. Semin Thromb Hemost 31: 174-183.

Shihmanter R, Nulman I, Goland S, Caspi A, Bar-Haim A, Harary I, Berkovitch M, Arcavi L (2014) Variation in the CYP2D6 genotype is not associated with carvedilol dose changes in patients with heart failure. J Clin Pharm Ther 39: 432-438.

Small DS, Farid NA, Payne CD, Konkoy CS, Jakubowski JA, Winters KJ, Salazar DE (2010) Effect of intrinsic and extrinsic factors on the clinical pharmacokinetics and pharmacodynamics of prasugrel. Clin Pharmacokinet 49: 777-798.

Su J, Xu J, Li X, Zhang H, Hu J, Fang R, Chen X (2012) ABCB1 C3435T polymorphism and response to clopidogrel treatment in coronary artery disease (CAD) patients: a meta-analysis. PLoS One 7: e46366, doi:10.1371/journal.pone.0046366

Tamura S, Tokunaga Y, Ibuki R, Amidon GL, Sezaki H, Yamashita S (2003) The site-specific transport and metabolism of tacrolimus in rat small intestine. J Pharmacol Exp Ther 306: 310-316.

Taubert D, von Beckerath N, Grimberg G, Lazar A, Jung N, Goeser T, Kastrati A, Schömig A, Schömig E (2006) Impact of P-glycoprotein on clopidogrel absorption. Clin Pharmacol Ther 80: 486-501.

Topol EJ, Schork NJ (2011) Catapulting clopidogrel pharmacogenomics forward. Nat Med 17: 40-41.

Tsume Y, Mudie DM, Langguth P, Amidon GE, Amidon GL (2014) The biopharmaceutics classification system: subclasses for in vivo predictive dissolution (IPD) methodology and IVIVC. Eur J Pharm Sci 57: 152-163.

Varma MVS, Sateesh K, Panchagnula R (2005) Functional role of P-glycoprotein in limiting intestinal absorption of drugs: contribution of passive permeability to P-glycoprotein mediated efflux transport. Mol Pharm 2: 12-21.

Varma MV, Gardner I, Steyn SJ, Nkansah P, Rotter CJ, Whitney-Pickett C, Zhang H, Di L, Cram M, Fenner KS, El-Kattan AF (2012) pH-dependent solubility and permeability criteria for provisional biopharmaceutics classification (BCS and BDDCS) in early drug discovery. Mol Pharm 9: 1199-1212.

Wang Z-Y, Chen M, Zhu L-L, Yu L-S, Zeng S, Xiang M-X, Zhou Q (2015a) Pharmacokinetic drug interactions with clopidogrel: updated review and risk management in combination therapy. Ther Clin Risk Manag 11: 449-467.

Wang X-q, Shen C-l, Wang B-n, Huang X-h, Hu Z-l, Li J (2015b) Genetic polymorphisms of CYP2C19*2 and ABCB1 C3435T affect the pharmacokinetic and pharmacodynamic responses to clopidogrel in 401 patients with acute coronary syndrome. Gene 558: 200-207.

Xie HG, Zou JJ, Hu ZY, Zhang JJ, Ye F, Chen SL (2011) Individual variability in the disposition of and response to clopidogrel: pharmacogenomics and beyond. Pharmacol Ther 129: 267-289.

Yu LX, Amidon GL, Polli JE, Zhao H, Mehta MU, Conner DP, Shah VP, Lesko LJ, Chen M-L, Lee VHL, Hussain AS (2002) Biopharmaceutics classification system: the scientific basis for biowaiver extensions. Pharm Res 19: 921-925.