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In vitro interaction of clopidogrel and its hydrolysate with OCT1, OCT2 and OAT1

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ABSTRACT

Clopidogrel (CP) is metabolized by CYPs to the active metabolite, or hydrolyzed by esterase to clopidogrel carboxylate (CPC) in liver, and CPC is partly excreted from urine. Therefore, the objective of the present study was to evaluate the interactions of CP and CPC with organic cation transporter 1 (OCT1) (in liver), and CPC with organic cation transporter 2 (OCT2) and organic anion transporter 1 (OAT1) (in kidney). Both CP and CPC inhibited the uptake of 1-methyl-4-phenylpyridinium (MPP^+) and metformin, typical substrates of OCT1, in MDCK-hOCT1 cells with low IC_{50} (0.307–14.0 μM). CPC (100 μM) reduced the uptake of MPP^+ and metformin mediated by OCT2 in MDCK-hOCT2 cells to 60.8% and 33.6% of the control, CPC (500 μM) decreased the uptake of 6-carboxyfluorescein (6-CFL) and para-aminohippuric acid (PAH), substrates of OAT1, in MDCK-hOAT1 cells to 64.6% and 79.4% of the control. CP and CPC were also found to inhibit other drugs of OCT1 substrates, such as lamivudine and amantadine, in MDCK-hOCT1 cells with the IC_{50} of 1.97–4.15 μM, except CPC on amantadine (IC_{50} > 100 μM). The inhibition of CP and CPC on lamivudine uptake in primary rat hepatocytes was also confirmed with the IC_{50} of 2.91 and 1.25 μM, respectively. Additionally, CP and CPC were not substrates of OCT1 and OCT2, whereas CPC was a substrate of OAT1 with the K_m of 5.61 μM. In conclusion, CP and CPC are strong inhibitors of OCT1, but weak inhibitors of OCT2 and OAT1, and CPC is a high affinity substrate of OAT1.

1. Introduction

Clopidogrel (CP) is a potent antiplatelet drug which has been widely applied for the reduction of atherosclerotic events in patients with stroke, myocardial infarction, cardiovascular disease, and acute coronary syndrome (Diener et al., 2005; Fox and Chelliah, 2007). A small proportion of administered CP is metabolized in liver by CYPs, especially by CYP3A4/5 and CYP2C19 (Kazui et al., 2010), and the formative metabolite exhibits an anti-aggregatory effect (Savi et al., 2000). The majority of CP is hydrolyzed to an inactive metabolite, clopidogrel carboxylate (CPC) by human carboxylesterase 1 (CES1) (Zhu et al., 2013). CP is absorbed rapidly and reaches the C_{max} of the parent molecule, active metabolite, and inactive CPC within 1 h after oral administration (Mullangi and Srinivas, 2009). Since more than 85% of CP is metabolized by hepatic esterases to CPC in human (Tang et al., 2006), CPC has been used as a surrogate marker for bioavailability of CP (Hagihara et al., 2009; Morjani and Madoulet, 2010). Although only a small fraction of CPC is excreted in urine after oral administration (Caplain et al., 1999), CPC can be detected in human plasma even at 48 h after dose, whereas CP itself is undetectable after 12 h of administration (Mitakos and Panderi, 2004).

The drug–drug interaction (DDI) mediated by metabolic enzymes and transporters is important for clinical medication, several CP–drug interactions based on liver CYPs have been reported. For instance, a potential interaction between proton pump inhibitors (PPIs) and CP was observed in clinical treatment (Pezalla et al., 2008; Juurlink et al., 2009), the most potential mechanism proposed is that both PPI and CP are metabolized by CYP2C19 (Li et al., 2004). Similarly, the CYP3A4-mediated metabolism is also the important pathway of CP disposition, the interaction between CP and substrates of CYP3A4, such as atorvastatin, was also observed (Clarke and Waskell, 2003). These interactions result

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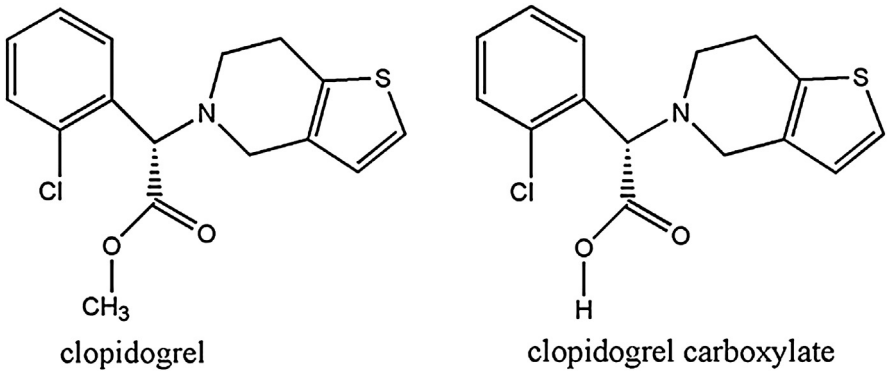

Fig. 1. Chemical structures of clopidogrel and clopidogrel carboxylate.

in decreasing the conversion of CP to the active metabolite, and subsequently reduce the effect of CP (Lau et al., 2003). It was also found that the absorption of CP and formation of active metabolite were reduced by P-glycoprotein (P-gp)-mediated efflux (Taubert et al., 2006), and P-gp inhibitors, such as quercetin, telmisartan, or cyclosporine, would influence the absorption of CP. An inhibition of CP absorption mediated by intestinal P-gp was confirmed *in vivo* (Lee et al., 2012). Although CP is mainly activated and inactivated in liver, and a part of CPC is excreted through kidney, CP/CPC–drug interactions mediated by liver or renal transporters have not been reported.

Organic cation transporter 2 (OCT2) and organic anion transporter 1 (OAT1) are highly expressed in human kidney (Nozaki et al., 2007), while organic cation transporter 1 (OCT1) is primarily located in human liver (Koepsell et al., 2007). OCTs mediate the cellular transport of endogenous and exogenous organic cations, while OATs mediate the transport of organic anions. Based on the structures of CP and CPC (Fig. 1), we deduced that they might interact with OCTs or OATs. Therefore, the present study is to confirm whether CP and CPC are inhibitors/substrates of OCTs and OAT1, in Madin-Darby canine kidney (MDCK) cells stably expressing human OCT1, OCT2 or OAT1, respectively. Subsequently, the interactions with some clinical drugs, such as lamivudine and amantadine, were evaluated in cell lines transfected with hOCT1 and in rat primary hepatocytes. The results of this study will help us to learn the potential possibility of transporters-mediated DDI for CP and CPC.

2. Materials and methods

2.1. Materials

CP (purity ≥98%) and CPC (purity ≥98%) were purchased from National Institutes for Food and Drug Control (Beijing, China) and J&K Scientific Ltd. (Beijing, China), respectively. 1-methyl-4-phenylpyridinium (MPP^+), metformin, 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide (ASP^+), para-aminohippuric acid (PAH), lamivudine, amantadine, 6-carboxyfluorescein (6-CFL), quinidine, cimetidine and probenecid were provided by Sigma Chemical (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium and fetal bovine serum (FBS, Gibco) were purchased from Invitrogen (Carlsbad, USA). Bicinchoninic acid (BCA) protein assay kit was obtained from Beyotime Institute of Biotechnology (Beyotime, China). Acetonitrile was purchased from Tedia Company (Fairfield, TX, USA). All other reagents were analytical and/or HPLC grade.

2.2. Animals

Male Sprague-Dawley rats, 180–200 g, were obtained from the Experimental Animal Center of the Zhejiang Academy of Medical Sciences. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang University. The rats were maintained in cages at controlled temperature (20–23 °C) and humidity (50–60%) with 12:12 h light–dark cycles and a free access to food and water. Animals were fasted for 12 h before experiment.

2.3. Cell culture

Madin-Darby canine kidney (MDCK) cell line was obtained from Peking Union Medical College (Beijing, China). MDCK cells stably transfected with plasmid pcDNA3.1 (+) vector containing human OCT1 cDNA (MDCK-hOCT1), human OCT2 cDNA (MDCK-hOCT2) and human OAT1 cDNA (MDCK-hOAT1) were constructed as reported previously (Shu et al., 2003; Urakami et al., 2004; Ho et al., 2000). MDCK cells were also transfected with empty vector pcDNA3.1 (+) as mock cells. The activity of hOCT1, hOCT2 and hOAT1 in the stably transfected MDCK cells were validated by model substrates, ASP^+ and MPP^+ for hOCT1 (Tu et al., 2013) and hOCT2, 6-CFL and PAH for hOAT1, respectively (unpublished data). Cells were grown in DMEM containing 10% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C with 5% CO_2 and 95% humidity. The cells were sub-cultured after being 90% confluent.

2.4. Primary rat hepatocytes isolation and culture

Primary rat hepatocytes were isolated from rat liver by a modified two-step collagenase perfusion method (Lu et al., 2011). The viability of the isolated hepatocytes was validated by Trypan blue uptake, and the cells with more than 85% viability were applied to the experiment. The cells were maintained in RPMI 1640 medium supplemented with 10% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin and then seeded in poly-d-lysine-coated 24-well plates (Costar Corning Inc., NY, USA) at a destiny of 2×10^5/well. The cells were cultured at 37 °C with a 5% CO_2 and 95% air humidified atmosphere for 4 h to be attached to the plates.

2.5. Cellular uptake study

Cellular uptake of CP, CPC or other substrates of transporters studied were performed in MDCK-hOCT1, MDCK-hOCT2, MDCK-hOAT1 cells and their mock cells as the method we reported previously (Tu et al., 2013). Cells were seeded in 24-well plates at a destiny of 2×10^5/well, on day 3 after seeding, cells were pre-incubated with Hank's balanced salt solution (HBSS, pH 7.4) for 5 min at 37 °C, and then 200 μL of HBSS containing CP, CPC, or other substrates was added to initiate the uptake in the absence or presence of inhibitors, the incubation was performed at 37 °C for the designated uptake time. At the end of the incubation, cells