เอนไซม์คาร์บอกซิลเอสเทอร์เรส 1: โครงสร้าง ความหลากหลายทางพันธุกรรม และบทบาทในการกระตุ้นและการย่อยยา

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บทคัดย่อ

เอนไซม์คาร์บอกซิลเอสเทอร์เรส 1 ในมนุษย์มีการแสดงออกอย่างมากที่ตับซึ่งเป็นเนื้อเยื่อที่เกิดปฏิกิริยาไฮโดรไลติกมาก เชื่อกันว่าเอนไซม์คาร์บอกซิลเอสเทอร์เรส 1 นี้ทำหน้าที่ในการเร่งการเกิดปฏิกิริยาไฮโดรไลติกประมาณ 80% ของปฏิกิริยาทั้งหมดที่เกิดขึ้นที่ตับ ส่วนที่เหลืออีก 20% นั้นจะเป็นหน้าที่ของเอนไซม์คาร์บอกซิลเอสเทอร์เรส 2 สำหรับเอนไซม์คาร์บอกซิลเอสเทอร์เรส 1 นั้นมีบทบาทสำคัญในการสลายสารตั้งต้นที่มีพันธะเอสเทอร์และเอไมด์ ซึ่งรวมถึงยาชนิดต่างๆ สารเอนโดไบโอติกส์ และสารซีโนไบโอติกส์ จากบทบาทความสำคัญของเอนไซม์คาร์บอกซิลเอสเทอร์เรส 1 ทำให้มีการศึกษาเกี่ยวกับเอนไซม์ชนิดนี้ในด้านต่างๆ เป็นจำนวนมาก เช่น โครงสร้างของยีนและความหลากหลายทางพันธุกรรม โครงสร้างของโปรตีนและบทบาทหน้าที่ในการทำงาน เป็นต้น นอกจากนี้ พบว่าเมื่อมีการเปลี่ยนแปลงลำดับของเบสเพียง 1 เบสหรือมีสนิปส์ในส่วนของยีน ในบริเวณโปรโมเตอร์ และในส่วนที่ไม่แสดงออกทางปลาย 5’ ของยีนจะส่งผลต่อการทำงานของเอนไซม์ ซึ่งการเปลี่ยนแปลงของการทำงานของเอนไซม์นี้ส่งผลกระทบต่อการรักษาทางคลินิก บทความฉบับนี้จะเน้นถึงความสำคัญของโครงสร้างของโปรตีนในด้านที่เกี่ยวกับการทำงานของเอนไซม์คาร์บอกซิลเอสเทอร์เรส 1 และความหลากหลายทางพันธุกรรมหรือการเปลี่ยนแปลงลำดับของเบสในแง่ที่ส่งผลกระทบต่อการทำงานที่เกี่ยวข้องกับการย่อยยา

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Human liver carboxylesterase 1
structure, polymorphism and its role in drug and prodrug activation and metabolism

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Abstract

The human carboxylesterase 1 or CES1 is an enzyme predominantly expressed in the liver where numerous hydrolytic reactions take place. It is believed that this enzyme is responsible for approximately 80% of hydrolytic activity in the liver and leaves the rest of 20% to its counterpart enzyme, human carboxylesterase 2 (CES2). CES1 plays a critical role in hydrolysis of numerous compounds which contain ester- and amide-bonds, including drugs, prodrugs, endobiotics, and xenobiotics. Owing to its significant role, extensive studies have been carried out to investigate its gene structure and polymorphisms, protein structure and function as well as many other aspects. The present review highlights the importance of the structure of CES1 in regard to its function and catalytic activity. Additionally, it was found that CES1 catalytic activity can be influenced by variations or SNPs in DNA coding region, promoter region as well as 5' untranslated region of CES1. These alterations in the catalytic activity of an enzyme have been shown to associate with clinical outcomes. Therefore, polymorphisms related to functional activity of CES1 in the hydrolysis of drugs and prodrugs are also discussed.

Keywords: CES1, structure, polymorphisms, drug and prodrug metabolism

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Gene structure and classification of Carboxylesterases

Mammalian carboxylesterases (CESs) are members of the serine hydrolase superfamily which catalyze the hydrolysis of amide, ester, and carbamate bonds. They are found in various mammals and exhibit broad substrate specificity. Carboxylesterases play an important role in the metabolism of numerous compounds, including xenobiotics and endobiotics. CESs can be classified into six families, namely CES1-CES6, according to their sequence identity in which members in the same family share sequence identity of 60% or higher. Among these families, CES1 is the largest family, containing 8 subfamilies[1]. On the other hand, based on the molecular and bioinformatics data, human genome comprises seven distinct carboxylesterase genes. They are assigned to CES1, CES2, CES3, CES5, and CES6 families and found to share sequence identity of 39–46%[2,3]. CES1 is predominantly expressed in the liver but is also found in various tissues including heart, testis, macrophages, and lung and is responsible for drug and prodrug metabolism and activation including endobitotics and xenobiotics hydrolysis[4-6]. CES1 has three members which are CES1A1, CES1A2, and CES1A3. Only CES1A1 and CES1A2 are functional where CES1A3 is an alternate form of CES1A2 containing a premature stop codon[7,8]. The difference between CES1A1 and CES1A2 is found in the signal peptide region where four amino acids are divergent, therefore, both CES1A1 and CES1A2 encode identical mature proteins[7]. On the other hand, CES2 is mainly expressed in intestine and has been demonstrated to be efficient in the hydrolysis of various compounds such as anticancer prodrug and cocaine[9]. Although both CES1 and CES2 are major hydrolytic enzymes responsible for hydrolysis of several therapeutic compounds, they differ in substrate preferences. CES1 favours small alcohol and large acyl group substrate whereas CES2 prefers substrate with large alcohol and small acyl group[10]. The third human carboxylesterase, CES3, is expressed in several tissues particularly colon, trachea, and brain tissue. Scant information is however available regarding the characterization and substrate specificity of this CES3. It is believed that CES3 participates in colon and neural drug metabolism[11]. CES5 is mainly expressed in peripheral tissues, including kidney, testis, brain, and lungs. It is a secreted protein and its function is to regulate the pheromone precursor production and to participate in lipid and cholesterol transfer processes[12]. The final member of human carboxylesterse, CES6, also known as CES4A also encodes a secreted enzyme that is responsible for detoxification of drugs and xenobiotics in neural and other tissues[13].

Structure of human CES1

CES1 belongs to an α/β-hydrolase-fold protein which contains a unique arrangement of alternate α-helix and β-sheets connected by loops with various lengths[14]. It shares similar structure to other members of α/β-fold enzymes such as acetylcholinesterase[15]. Currently, five crystal structures of human CES1 in complex with different compounds have been determined[16-20]. Overall, the crystal structures of CES1 exhibited an α/β-hydrolase-fold in which one monomer is formed by 17 α-helices and 20 β-strands, consisting of three domains which are central catalytic domain, surrounding by an $\alpha\beta$ domain and a regulatory domain. The serine hydrolase catalytic triad consisting of Ser221, Glu354, and His468 is located at the base of the active site gorge in the central catalytic domain of the protein which is composed of strands β1–9, β12–13, and β16–19 and helices α1–5, α9 and α13–15. The $\alpha\beta$ domain contains α6–8, β10–11 and β14–15 while regulatory domain consists of α10–12, α10', and α16. It was also shown in the structure that CES1 has a conserved N-linked glycosylation site at Asn79 which is believed to help in protein folding, solubility, and trimer stabilization. Moreover, glycosylation at Asn79 is also important for the function of the enzyme[18].

Additionally, the crystal structures have indicated that CES1 enzyme actually contains three ligand binding sites which are the active site, side door, and Z-site. The active site is settled at the bottom of a catalytic gorge and aligned in a serine esterase typical character[19]. In fact, the substrate-binding gorge of the enzyme consists of two pockets; one is large and flexible located on the one side of Ser221 while the other one is rather small and rigid sitting on the opposite side. The

larger binding site provides flexibility as well as comfort to promote the binding towards various substrates for the enzyme, causing the promiscuous activity of CES1. The second ligand binding site, the side door, is a secondary pore that leads into the enzyme's active site from the exterior, located adjacent to the large and flexible substrate-binding pocket. It has been proposed that this opening allows the direct access of ethanol to the acyl-enzyme intermediate during the transesterification of cocaine. Therefore, the function of this side door is to shuttle small molecules into and out of the active site of the enzyme. Finally, the Z-site located on the surface of the enzyme, is crucial for trimer-hexamer equilibrium of the enzyme[16]. In hexamer form, two trimers are stacked with their substrate-binding gorge facing in, an creating extensive dimer interface which results in interdigitation of two loops (Ω1 and Ω2) and creates a Z-shape dimer interface or Z-site[16,17]. It was found during olimerization analysis of CES1 using atomic force microscopy (AFM) that the presence of ligand analogue, homatropine, moves the trimer-hexamer equilibrium toward the trimer form. With the ligand bound in Z-site, the site is, therefore, not available for interacting with another trimer, hindering the packing of two trimers to form a hexamer. Consequently, binding of ligand to Z-site causes the shift of the trimer-hexamer equilibrium and Z-site is accessible for the ligand only when enzyme is in the trimer form[17]. The movement of equilibrium toward trimer form apparently promotes the binding of substrate to the enzyme binding site as well as enhances catalysis. It has been observed that various types of ligands could bind to the Z-site of CES1, suggesting the promiscuity of this site. This feature also reflects the promiscuous activity of the enzyme as CES1 is able to catalyze a wide variety of substrates. Furthermore, the structural analysis and kinetic data of CES1 have suggested that Z-site is also important for the function of the enzyme in which this site plays direct role in the allosteric activation of catalysis.

Role of CES1 in drug, prodrug metabolism, and activation

CES1 is predominantly expressed in the liver where it plays important role in the metabolism of a wide variety of compounds containing ester bonds, including drugs, prodrugs, and xenobiotics[21,22]. Additionally, CES1 is known to catalyze endobiotics such as those associated with cholesterol and fatty acid homeostasis[23]. In fact, CES1 favourably hydrolyses substrate with small alcohol group and a large bulky acyl group which is in contrast to CES2 where a large alcohol and small acyl group is preferred. In this review, metabolism and activation of drug and prodrug by CES1 will be focused.

Immunosuppressant agents

Inosine monophosphate dehydrogenase (IMPDH) is the rate-limiting enzyme in de novo pathway of guanine nucleotide biosynthesis. This enzyme is important for DNA, RNA, and glycoprotein synthesis, cell signalling pathway, including processes involved in cellular proliferation. The enzyme has therefore been proposed as a drug target for immunosuppressive and cancer chemotherapy. Mycophenolic acid (MPA), an immunosuppressant agent, is the active metabolite of ester prodrug, mycophenolate mofetil (MMF). It is a noncompetitive inhibitor of IMPDH. Fujiyama et al. have studied the hydrolysis of MMF by CES1 and CES2 and found that both human carboxylesterase can hydrolyse this ester prodrug, resulting in an active metabolite MPA[24]. However, CES1 catalysed the reaction approximately 10-fold faster than that of CES2, suggesting that CES1 is the main enzyme responsible for the activation of MMF prodrug in the liver. Since MMF is orally administered and encountered the intestine before the liver, MMF hydrolysis by CES2 might have occurred as well. Therefore, the detailed study of both enzymes regarding MMF metabolism is required and would be important to provide useful information for understanding the pharmacokinetics of this drug.

Anaesthetics

There has been reported that CES1 can hydrolyse amide-type local anaesthetics, prilocaine and lidocaine[25]. The lidocaine/prilocaine combination (equal in weight) is used to prevent pain associated with intravenous catheter insertion, blood collection, and superficial surgical procedures. The mixture is commonly used in dentistry. Hydrolysis of prilocaine results in

the aromatic amine o-toluidine while metabolism of lidocaine gives 2, 6-xylidine. Although both metabolites are suspected to be involved in methaemoglobinemia. Lidocaine/prilocaine combination however is still safe to use in patients. This study has provided evidence regarding the importance of CES1 in drug toxicity.

Antihypertensive agent

Prodrug trandolapril is an inhibitor of the angiotensin-converting enzyme (ACE) and is currently used in clinical treatment of hypertension. Trandolapril, itself, is a weak ACE inhibitor and, therefore, requires a biotransformation to its active metabolite, trandoprilate[26]. It is believed that the bioactivation of trandolapril to its active metabolite occurrs in the liver. Trandoprilate illustrates a greater inhibition activity of approximately 8 folds against ACE when compared to its parent compound. In the study where hydrolysis of trandolapril was determined by *in vitro* incubation with human liver microsomes where CES1 is abundantly expressed and human intestine microsomes where CES2 is predominant, it was found that trandolapril was hydrolysed only by liver but not intestine microsomes. Additionally, cell lines stably expressing native CES1 was also efficiently catalysed trandolapril. This suggested that CES1 is responsible for trandolapril metabolite activation[27].

Antiplatelet agent

Adenosine-5'-diphosphate (ADP) is an important mediator in metabolism and also plays crucial role in energy flowing in living cells. One of its roles is to be a mediator in blood platelet activation. ADP is stored inside blood platelet and released upon platelet activation. In this situation, it is acting as primary mediator by interacting with ADP receptors on platelet which leads to platelet activation. Therefore, inhibition of platelet aggregation is critical for treatment of arterial occlusive disease, especially for managing patients with coronary artery disease and acute coronary syndrome (ACS), including patients undergoing percutaneous coronary intervention (PCI)[28,29].

Thienopyridine prodrugs such as ticlopidine, clopidogrel and prasugrel, are inhibitors of adenosine-5'-diphosphate (ADP)-mediated platelet aggregation *in vivo*[30,31]. Combination of aspirin and clopidogrel is widely used as antithrombogenic agents and is proven to be successful for treatment and prevention of cerebro- and cardio-vascular problems[32-34]. It was first shown by Tang et al. that human CES1 was an enzyme responsible for the hydrolysis of clopidogrel and its metabolite was identified. On the other hand, aspirin was mainly hydrolysed by CES2. Though both CES1 and CES2 are capable of hydrolysing the transformation of prasugrel to its metabolite, R95913, the catalysis rate of CES2 seems to be greater than that of CES1 for 25-fold, suggesting that CES2 is mainly responsible for bioactivation of prasugrel[35].

Psychostimulants

Methylphenidate (MPH) is commonly considered as "gold standard" for managing attention-deficit/hyperactivity disorder or ADHD. It was found that there is a difference of each individual in regarding the metabolism and disposition of two enantiomers of MPH, l- and d-MPH[36]. The metabolic pathway governing the metabolism of MPH is deesterfication which is mediated by CES1 in an enantioselective manner and hydrolysis of the l-isomer is favoured over d-isomer[37]. However, pharmacokinetics analysis of MPH has indicated that l-isomer is only accounted for small amount of MPH circulated in blood while d-MPH is the main species. Zhu et al., have reported the enzymatic activity of CES1 towards the hydrolysis of p-nitrophenyl acetate (pNPA), d-MPH and l-MPH. It was found that WT CES1 demonstrated notable activity on pNPA hydrolysis. In addition, MPH metabolism by WT CES1 showed the significant stereospecific with l-MPH favored over d-isomer[38]. These stereoselective manners of CES1 were in agreement with previous observations[39]. This indicated that CES1 is an enzyme responsible for metabolism of MPH.

Antiviral agents

CES1 also plays important role in anti-human HIV prodrug activation. This was evident from the study by Saboulard et al. in which the metabolism of phosphoramidate triester prodrugs of stavudine and zidovudine mediated by carboxylesterase was

investigated[40]. Both stavudine and zidovudine are nucleoside analogue reverse-transcriptase inhibitor (NARTI), active against HIV. They are analogue of thymidine which will be phosphorylated by kinase into its active triphosphate form. Triphosphate forms of stavudine and zidovudine will cause termination of DNA replication by competing with their natural substrate (thymidine triphosphate) during incorporation into the DNA strand. It has been shown that the first activation step of these two compounds is mediated by carboxylesterase[41-43]. Additionally, the efficiency of this step is dependent on the amino acid, alkyl ester and dideoxynucleoside moiety by which amino acid group has the most influence on pharmacokinetics of the triester as well as associates with the stability. It was also revealed that stability of ester derivative provides a small advantage in antiviral activity.

Oseltamivir phosphate, a neuramidinase inhibitor, is an ester prodrug which requires a conversion into its active metabolite, oseltamivir carboxylate, by CES1[44]. It is the main medicine recommended by the World Health Organization (WHO) and commonly used for the treatment of influenza virus A and B infections[45]. Since the biotransformation of many compounds predominantly occurs in the liver it was proposed that carboxylesterases are responsible for this action. In a clinical study, it was found that the hydrolysis of oseltamivir was extremely slow in children who express low levels of carboxylesterase enzymes[46-48]. It was proven later that the enzyme responsible for oseltamivir metabolism is CES1 rather than CES2. The in vitro assay using liver and intestine microsomes as well as recombinant CES1 and CES2 enzymes has shown that hydrolysis of oseltamivir only occurrs in the presence of liver microsomes and recombinant CES1 enzyme. While none of the active metabolite, oseltamivir carboxylate, was detectable for neither intestine microsomes nor recombinant CES2[44]. This strongly indicated that CES1 not CES2 is the enzyme responsible for the biotransformation of oseltamivir and that the liver is the primary organ where metabolism of oseltamivir happens. Furthermore, an investigation on the conversion of oseltamivir to its metabolite by using two variant enzymes has provided further evidence that CES1 is important for activation of oseltamivir[49].

Anticancer agents

One of the most significant roles of carboxylesterase in prodrug and drug biotransformation is its function in metabolism of anticancer. At the present, many anticancer agents have been investigated and CES1 was shown to be responsible for hydrolysis of several agents. Brivanib alaninate prodrug is being developed for anticancer treatment. It exerts its function by inhibiting vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) signalling pathway through the inhibition of tumour-induced angiogenesis[50,51]. It is currently in phase II/III clinical studies for the treatment of cancer[52]. Interestingly, brivanib, an active metabolite of brivanib alaninate, showed promising activity in both preclinical and clinical studies[53-55]. The hydrolysis of brivanib alaninate prodrug was studied and the results have shown that both CES1 and CES2 can convert brivanib alaninate into its active moiety[56]. However, it is still not possible to rule out that other liver esterases are also involved in the hydrolysis of this prodrug. Therefore, more information is required to examine which esterase is mainly responsible for brivanib alaninate activation.

It was known that cancer cells exhibited abnormal DNA methylation patterns which are also associated with aberrant silencing of tumour suppressor genes[57,58]. As a consequence, inhibitors of DNA methylation have been developed as anticancer treatment. Azacytidine (5-aza derivatives of cytosine) is a potential inhibitor of DNA methyltransferase and it has been going through development to obtain a better anticancer activity[59]. Fortunately, it was found that modification of N4 position of the azacitidine ring (NPEOC-DAC) can be used to inhibit DNA methyltransferase and is more effective than its parent compound, azacitidine, at inhibiting DNA methylation at high concentration e.g. more than 10 μM. The study by Byun et al., has indicated that the anticancer activity of NPEOC-DAC is limited to cells expressing CES1[60]. This indicates that carboxylesterase is responsible for the cleavage of the N4 carboxylester bond, converting NPEOC-DAC to its active metabolite, decitabine.

Among anticancer agents, irinotecan or CPT-11, a clinically approved anticancer drug, is

one of the most widely investigated agent[61]. Though conversion of CPT-11 to its active metabolite SN-38 has been elucidated in mammal species, enzymes responsible for the conversion in human were not clearly identified[62]. Previous reports have shown that tumour cells expressing human liver carboxylesterase can hydrolyze CPT-11, giving SN-38[63-65]. In 2000, purified human liver carboxylesterase enzymes (CES1 and CES2) were investigated for the bioactivation of CPT-11[66]. It was found that both CES1 and CES2 were able to convert CPT-11 to SN-38, however, CES2 showed a greater catalytic activity towards the hydrolysis. This was in agreement with other studies where CES2 have considerably greater potential for the conversion of CPT-11 to SN-38[67]. It was later investigated that the descent activity of CES1 resulted from the size of the active site entrance. With small active site entrance of CES1, it cannot accommodate the bulky substrate like CPT-11 well when compared to other carboxylesterases such as rabbit and intestine CES or CES2[68]. Modelling of the kinetic data for bioactivation of CPT-11 fit a two-enzyme model with a high- and low-affinity isoforms of liver carboxylesterases. The role of CES1 in hydrolysis of CPT-11 is therefore still unclear.

Capecitabine, prodrug of 5-fluorouracil, is an approved treatment of metastatic breast and colorectal cancers[69]. It undergoes a three-step activation process in which the first step is the hydrolysis of carbamate side chain of capecitabine, producing 5'-deoxy-5-fluorocytidine (5'-DFCR). This step occurs primarily in the liver by carboxylesterases[70-73]. However, a specific enzyme responsible for capecitabine hydrolysis has not been unequivocally described. Quinney et al. have reported the steady-state kinetics analysis of capecitabine by purified human carboxylesterase isozymes (CES1A1, CES2, and CES3)[74]. Both CES1A1 and CES2 showed promising activity towards capecitabine hydrolysis while CES3 exhibited extremely low activity. Nonetheless, it was found that catalytic efficiency of CES1A1 for capecitabine hydrolysis was slightly greater than that of CES2. Earlier, it has been reported that biotransformation of capecitabine by crude lysate prepared from human liver was 19-fold more efficient than that prepared from human intestine[71]. It is clearly seen that activation of capecitabine mainly occurred in the liver and it is likely that CES1 is predominantly responsible for capecitabine hydrolysis. However, more evidences are needed to confirm this hypothesis e.g. the hydrolysis efficiency of CES2 towards capecitabine hydrolysis in the intestine.

Association between CES1 polymorphism and its role in drug/prodrug metabolism

In addition to study the role of CES1 in drug and prodrug metabolism, investigation of the clinical significance of CES1 genotypes is also critical to fully understand its function. This is owing to the fact that alterations in carboxylesterase sequences could result in an uncertainty in drug metabolism of each individual patient. Currently, there are total of 112 variations in CES1 gene reported in the NCBI database of single-nucleotide polymorphisms (dbSNP)[75]. Among them, there are 34 synonymous and 78 nonsynonymous SNPs. Since most of SNPs occurred are nonsynonymous it also suggests that these nucleotide alterations might have an effect on the enzymatic activity of CES1. Apparently, SNPs were not only found in the coding region of CES1 but also in introns, 5'-unstranslated region as well as promoter region.

Previously, it was found that transcriptional level of CES1 increases when there were variations in exon 1, indicating that polymorphisms in the upstream region might associate with the expression levels. In the study of Japanese hypertensive populations, it was observed that SNP at position -816 (promoter region, A>C) of CES1 had influenced the reduction in blood pressure when imidapril was used in the treatment[76]. This indicates that this SNP is important for promoter activity of the gene in which it has an effect on the transcriptional activity, enhancing CES1 expression as well as imidapril efficacy. Additionally, SNP at position -816 was found to be associated with several other SNPs in the proximal promoter regions, generating two additional binding sites for Sp1 (specificity protein 1 transcriptional factor). It was suggested that these two extra Sp1 binding sites are associated with increase transcription of CES1[77].

Though it was found that CES2 activity toward anticancer CPT-11 is much higher than that of

CES1[61,66], the common SNPs of CES2 found in Japanese population does not show any significant effects on irinotecan pharmacokinetics. The previous report on CES2 polymorphisms in Japanese population has indicated that there were only minor genetic variations which were associated with lower expression/function of CES2 *in vivo* and *in vitro*[78,79]. Since CES1 is significantly expressed in the liver where drug and prodrug metabolism occurs, therefore, it is likely that genotypes of CES1 might associate with plasma concentration of metabolites, including SN-38, the active metabolite of anticancer irinotecan. A pharmacogenomic analysis of both CES1 and CES2 in 120 people revealed 16 SNPs in CES1 gene by which 2 of them were nonsynonymous, other 2 were SNPs in the 5'-unstranslated region and 12 were intronic SNPs (80). No significant association between CES1 polymorphisms and RNA expression was observed. However, it would be necessary to perform functional analysis of these known polymorphisms because it will provide useful information regarding their roles in protein expression and enzymatic activity.

A study in cancer patients led by Sai et al. also in Japanese populations, has detected four other novel variations, one in the 5'-unstranslated region and three in 5'-flanking region of CES (-285C>T, -233C>A, -161A>G, -30G>A) (81). The effects of SNPs on irinotecan metabolism were examined. It was found that -75G>T SNP increased the AUC ratio while no significant impact of -30G>A was observed. The frequency of other three novel SNPS was quite low, making it difficult to assess the statistical analysis. This would require large samples to obtain the clinical importance of CES1 genotyping in irinotecan treatment. Interestingly, the in vivo CES activity was expanded depending on the number of functional CES1 genes, indicating a partial but critical role of CES1 on activation of irinotecan.

The effect of CES1 genetic polymorphisms on the pharmacokinetics of oseltamivir, an anti- influenza prodrug, was also examined in healthy Japanese male and female by Suzaki et al[82]. It was found that CES1 genotypes had no significant effect on the pharmacokinetic parameter of oseltamivir. In addition, there was no correlation between number of functional CES1 genes and oseltamivir metabolism. It should be noted that CES1 variants in studied were alterations in exon 1 only; therefore, it is still inconclusive to clarify the involvement of individual CES1 polymorphism in oseltamivir metabolism based on this study only. Also, it is possible that other proteins e.g. organic transporter 3 and multidrug resistance-association protein as well as other mutations in CES1 are associated with metabolism of oseltamivir. Previously, two nonsynonymous variants in CES1 coding region, Gly143Glu in exon 4 and a deletion in exon 6 resulting in frameshift mutation (Asp260fs) which altered the following 39 amino acids, have been identified and it was found that both mutations significantly altered the hydrolytic activity of CES1[83]. The supernatant collected from cell lines stably expressing native as well as two mutants were studied in which the hydrolysis of oseltamivir was assessed. It has been shown that the biotransformation of oseltamivir was remarkably impaired in both CES1 variants, Gly143Glu and Asp260fs. Reduction of about 75% in V_{max} and an increase of approximately 2-fold of K_M were observed for Gly143Glu polymorphism towards the hydrolysis of oseltamivir. Interestingly, Asp260fs mutant was unable to produce any detectable amount of oseltamivir metabolite using HPLC analysis, indicating the complete activity loss of this variant. The substantial decreased activity of both CES1 variants, especially Asp260fs, suggests the possibility of oseltamivir treatment failure. Fortunately, the prevalence of Asp260fs mutation was extremely low, none of the 925 subjects carried this mutation in CES1 genotypic analysis. Its effect on oseltamivir activation would therefore be only minor. However, cautions should have been taken during the treatment of influenza infections using oseltamivir.

In fact, two natural variants, Gly143Glu and Asp260fs, were identified during the investigation of the interaction of psychostimulant methylphenidate (MPH) and alcohol in 20 normal volunteers. One white male subject exhibited a highly abnormal concentration versus time, suggesting that his CES1 might be defective[83]. DNA sequencing of his CES1 gene has revealed two SNPs, Gly143Glu and Asp260fs. In a later study, the frequency of these two variants in specific racial and ethnic groups was determined[38]. It was observed that Gly143Glu is common with the frequency of 3.7%, 4.3%

and 2.0% in white, black, and Hispanic population, respectively. On the other hand, Asp260fs happens to be a rare mutation with a frequency significantly lower than 1% because none of 925 subjects exhibited this genotype. In addition, the catalytic activity of these two variant enzymes towards the hydrolysis of MPH was also assessed. Dramatically decrease catalytic activity was observed for both mutants towards the hydrolysis of pNPA. It was found that WT enzyme preferred to hydrolyze l-MHP isomer over d-MPH for about 10 times. Expectedly, both mutant enzymes showed no measurable catalytic activity toward MPH. These two identified variants have resulted in completely loss of CES1 activity and this may be the cause the poor response as well as adverse events in MPH treatment.

Other studies have been carried out to investigate the role of two CES1 variants, Gly143Glu and Asp260fs, in metabolism of different compounds. Incubation assay of cell lines stably expressing WT as well as natural mutants was performed for trandolapril and pNPA[27]. The hydrolytic activity of both variants was significantly decreased for the hydrolysis of pNPA. Gly143Glu exhibited only 30% activity when compared to the native CES1 while Asp260fs hardly catalysed the hydrolysis of pNPA substrate. Importantly, it was found that catalytic activity of both mutants towards trandolapril was undetectable, suggesting the significance role of these two SNPs relative to the clinical response and potential adverse effects associated with trandolapril pharmacotherapy.

Since there were reports of numerous CES1 polymorphisms from many laboratories as well as the database of SNP from NCBI, Tang et al., have assessed the influence of natural variants of CES1 on the hydrolysis of clopidogrel[34]. It was found that most of mutant enzymes tested in the study showed similar activity on the hydrolysis of clopidogrel to the native enzyme. Importantly, among 7 CES1 variants, only Cys70Phe exhibited lower expression level than other variants and also lost catalytic activity toward the hydrolysis of clopidogrel. Another study of clopidogrel hydrolysis by two CES1 variants (Gly143Glu and Asp206fs) has shown that these two mutants were completely inactive[84]. On the other hand, other natural variants, Gly18Val, Ser82Leu, and Ala269Ser have no significant effect on the hydrolysis of clopidogrel and its derivative, 2-oxo-clopidogrel. They suggested that two inactive mutants may be responsible for higher plasma concentration of clopidogrel-active metabolite and promote the antiplatelet activity. This indicates that genetic polymorphism of CES1 may relate to pharmocokinetics of clopidogrel and, as a consequence, the efficacy of its treatment.

Conclusions

Recently, enzymes responsible for drug and prodrug metabolism receive much attention from researchers. It is crucial that biotransformation of these compounds occur to produce the active metabolites otherwise these drugs would be unable to exert their pharmaceutical effects. Among numerous enzymes, carboxylesterases are in particular interest because it was found that approximately 80% of hydrolytic activity manifests in the liver and CES1 is responsible for that action while the rest of 20% belongs to CES2, mainly expressed in intestine. Owing to the critical role of CES1, its structure, function as well as polymorphisms associated with drug/prodrug metabolism have been extensively investigated. The structural studies of CES1 exhibited the α/β-hydrolase fold protein, composing three domains which are central catalytic, α/β and regulatory domains. The active site contains catalytic triad (Ser221, His468 and Glu354) located at the base of active site gorge. CES1 also has a conserved N-linked glycosylation at Asn79 which is believed to be important for its function. Additionally, its promiscuity towards various substrates was proposed to be the result of large and flexible binding site, providing the comfortableness for substrate binding. Polymorphism in genes encoding enzymes responsible for drugs/prodrug activation has a significant impact on patient treatment. If rare mutations are observed for the responsible enzyme, a predictable clinical response would be expected. However, if the genes show high mutation rate especially those associated with defective enzyme it would absolutely have an influence on drug metabolism. This could lead to therapeutic failure as well as unanticipated adverse effects or toxicities of the drugs. Fortunately for

CES1, most of the common variants studied so far still functional with in vitro catalytic activity assay showed comparable activity to that of WT enzyme. Exception has been made for two critical SNPs, Gly143Glu and Asp260fs, both of them demonstrated a significant decreased in hydrolysis towards various substrates e.g. pNPA, oseltamivir, MPH, and trandolapril. Gly143Glu, a common variant, is still able to catalyse the hydrolysis of drugs with remaining activity of approximately 25-30% when compared to the native enzyme. Though Asp260fs mutation results in completely loss of enzymatic activity, the frequency of this SNP is extremely low. Problems associated with clinical failure and adverse effects would therefore be only very minor. However, it would be of great importance to be able to screen the polymorphism before drug administration. Furthermore, functional analysis of each CES1 polymorphism will be useful to investigate its role in mRNA and protein expression as well as its catalytic activity. This investigation would be necessary to uncover the origin of distinct differences in drug and prodrug metabolism by variants of CES1. Nonetheless, it requires much work and effort.

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