

THE ROLE OF CARBOXYLESTERASE ENZYMES IN CAPECITABINE THERAPY

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ABSTRACT

INTRODUCTION: Capecitabine (CAP) is an oral antineoplastic pro-drug, whose initial step of activation is carboxylesterase (CES) dependent. The main conversion of CAP to 5-DFCR occurs in the liver by CES1 and a minor part - in the gastrointestinal tract (GIT) by CES2. Usually, the enteral pro-drug activation is associated with the appearance of fluoropyrimidine GIT toxicity, which may be dose-limiting and life-threatening for the patient. Thus, it is important to clear out the factors that could influence on the activity of both CES isozymes.

AIM: The aim of the present study was to present the mechanism of hydrolysis, sources of variability and modulation possibilities of CESs, that could affect the treatment with CAP.

MATERIALS AND METHODS: A systematic review of the scientific databases in PubMed, Science Direct and Google Scholar was conducted.

RESULTS: The literature data showed up to 89% inter-individual variability in the plasma content of CAP and its metabolites. It was also established that factors, such as genetic polymorphisms, age, gender, and diseases, are responsible for these variabilities. Enzyme inhibitors and inductors, on the other hand, are among the factors that could be controlled and used as reliable modulators for CAP therapy. In fact, some authors found that the inhibition of CAP hydrolysis at CES2 level could reduce the common GIT toxicity and improve the bioavailability of the pro-drug.

CONCLUSION: In accordance with the individual patient, the CES activity modulation approach could be used for the enhancement of the CAP therapeutic index. However, further detailed in vivo researches are needed to achieve categorical and applicable results.

Keywords: *capecitabine, carboxylesterases, CES1, CES2, therapeutic modulation*

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INTRODUCTION

Capecitabine (CAP, N4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine) is an oral antineoplastic pro-drug (1). Its development is a strategy for coping with the undesirable adverse effects of i.v. administered 5-fluorouracil (5-FU). The pro-drug design provides enhanced specificity toward cancer cells, reduction of toxicity, and additional treatment costs, respectively (2).

The activation of CAP includes three-step, enzymatically catalyzed process (3). The first step is a hydrolytic decomposition of the carbamate functional group by carboxylesterase enzymes. The product of this transformation is 5'-deoxy-5-fluorocytidine (5-DFCR). The second and third steps are oxidative deamination and de-deoxyribosylation reactions, catalyzed by cytidine deaminase (CD) and thymidine phosphorylase (TP) enzymes, respectively. The final product of this transformation is the cytostatic agent 5-Fluorouracil (5-FU) (Fig. 1) (4).

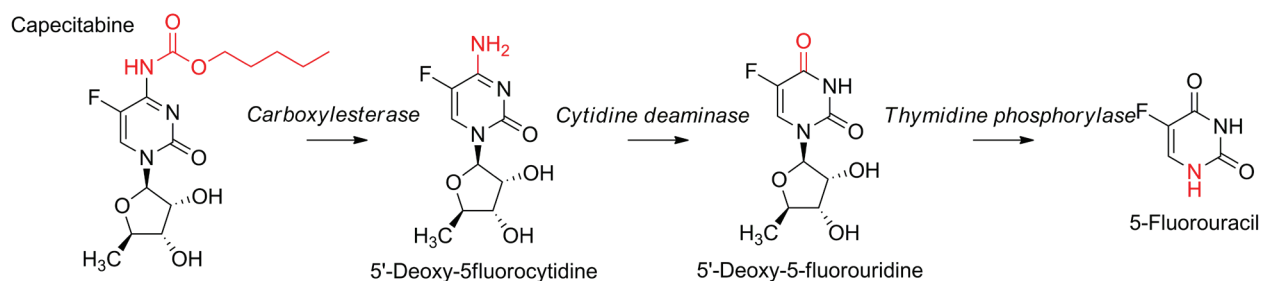


Fig. 1. A schematic representation of the three-step enzyme conversion of CAP

The skin, like other organs in the human body, As it was mentioned above, carboxylesterase (CES) enzymes are responsible for the initial step of CAP activation (2,3). They have been shown to be related to the efficacy and bioavailability of the pro-drug, as well as the manifestation of its toxicity (5).

Moreover, CESs are involved in the metabolism of over 10% of all clinically administered medicinal substances (6). Therefore, in recent decades, these enzymes have been a subject of interest in nu-

merous studies, concerning the biotransformation of drugs having ester, thioester, or carbamate functional groups, including the discussed here pro-drug.

The current study aims to present the mechanism of hydrolysis, sources of variability, and modulation possibilities of carboxylesterase enzymes, related to the treatment with CAP.

Carboxylesterase Superfamily

Carboxylesterase enzymes (CES, EU 3.1.1.1) are identified in species ranging from bacteria to hu-

mans (7,8). They are ubiquitous glycoproteins that belong to the α,β -serinehydrolase family (9). CESs are mainly expressed in tissues with a barrier function - epithelium of lungs and gastrointestinal tract (GIT), liver, kidneys, skin and other (10).

CESs play an important role in phase I of endobiotic and xenobiotic metabolism. Their substrates are a number of endogenous substances, medicines and toxins (Table 1) (11,12).

Table 1. Potential of bexarotene derivatives for the production of dermal metabolites

CES Substrates	
Drugs	aspirin, capecitabine, cilazapril, clopidogrel, cocaine, dabigatran etexilate, enalapril, heroin, imidapril, irinotecan, meperidine, methylphenidate, olmesartan, orlistat, oseltamivir, quinapril, ramipril, temocapril, trandolapril
Endogenous substances	acyl-CoA, acylcarnitine, triacylglycerol, cholesterol ester
Other	phthalates, benzoates, pyrethroids, pyrethrins, organophosphates, pesticides

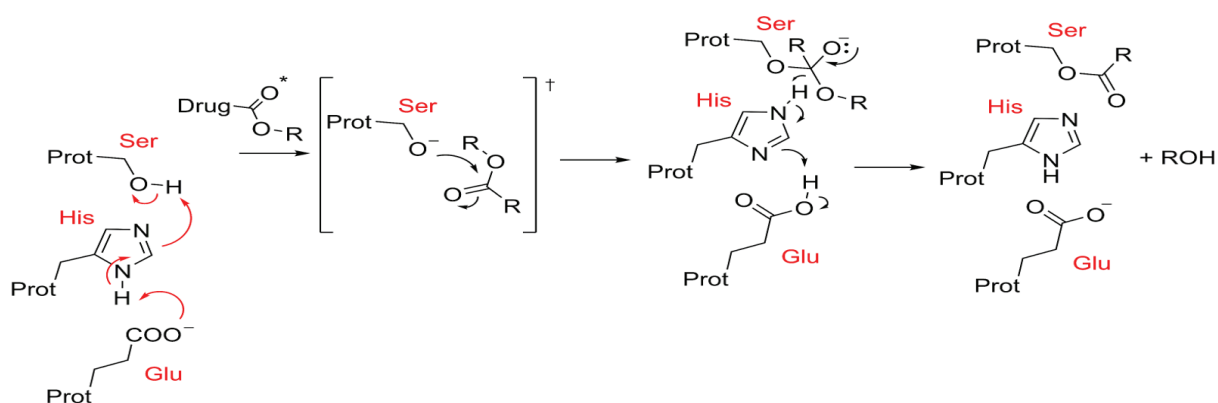


Fig. 2. CES-hydrolysis in humans

Carboxylesterase Mechanism of Hydrolysis

Carboxylesterase enzymes (CES, EU 3.1.1.1) are identified in species ranging from bacteria to humans (7,8). They are ubiquitous glycoproteins that belong to the α , β -serinehydrolase family (9). CESs are mainly expressed in tissues with a barrier function - epithelium of lungs and gastrointestinal tract (GIT), liver, kidneys, skin and other (10).

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Characteristics of Human Carboxylesterases

Human carboxylesterases are classified into 5 families: CES1, CES2, CES3, CES4, and CES5. CES1 and CES2 are found to be principally responsible for the biotransformation of xenobiotics (14). Despite their common origin, they share only 47-48% amino acid homology (15). The significant role of CESs in the metabolism and activation of multiple drugs requires elucidation of these specific features.

CES1 is expressed in the liver, adipocytes, and, to a lesser extent, in the kidneys, monocytes, lungs, GIT, testicles, heart, and macrophages. CES2 is mainly expressed in the GIT and less in the kidneys, liver, heart, brain, and testicles. For this reason, CES1 is also called hepatic (hCES), and CES2 is defined as human intestinal CES (hiCES) (16). It is supposed that the expression of CESs in the intestinal enterocytes impedes the absorption of the hydrolyzed substrates (6). Thus, from the perspective of drug metabolism, the administration of CES2 sensitive drugs should be considered with a particular caution.

A major characteristic of the detoxification properties of human carboxylesterase enzymes is their low substrate specificity (17). However, there are some differences established in both isozymes that could be summarized as follows: CES1 metabolizes predominantly small, planar ester substrates, with small alcohol groups and bulky acyl residues (enalapril, oseltamivir, imidapril, clopidogrel, mepredine, as well as the narcotic substances heroin and cocaine); CES2 has a greater tendency to hydrolyze esters with relatively larger alcohol groups and smaller acyl residues (irinotecan, prasugrel, flutamide, fluorescein diacetate) (16,18,19).

Role of CESs in Activity and Toxicity of CAP

The conversion of CAP to 5-DFCR occurs mainly in the liver (20). However, it should not be neglected that a measurable part of the orally administered CAP could also be degraded in the GIT by CES2, cytidine deaminase and thymidine phosphorylase enzymes (3). Therefore, CES2 should be considered as relevant to the first-pass metabolism and bioavailability of CAP (21).

Usually, the pro-drug activation in the gastrointestinal tract is associated with the appearance of fluoropyrimidine GIT toxicity (diarrhea and mucositis) (22, 23). It is often life-threatening or at least dose-limiting for the patient, and therefore requires additional medication.

Possible CES-Related Sources of Variability in CAP Plasma Exposition

Pharmacokinetic assays show high inter-individual variability (up to 89%) in the plasma content of CAP and its metabolites (24). This is consist-

ent with the observation that approximately 25% of patients experience severe adverse reactions (ADRs), such as hand-foot syndrome, diarrhea, anemia, nausea, vomiting, mucositis/stomatitis, and other (25, 26). In combination with the low therapeutic index of the pro-drug, it is difficult to evaluate the benefit/risk ratio for each patient (27). The identification of factors responsible for these deviations would enable the prediction of CAP bioavailability, as well as the risk of ADRs. This could be a basis for optimizing the therapeutic process (28,29).

Both isozymes (CES1 and CES2) are able to hydrolyze CAP to 5-DFCR. Thus, it is reasonable to summarize all endo- and exogenous factors that could affect their expression (30,31):

CES Gene Polymorphism in CAP Treatment

According to Vesell ES (2000) from 20% to 95% of the drug pharmacokinetic and pharmacodynamic variability is due to genetic factors (32). As a pro-drug that undergoes a three-step enzyme conversion, CAP is not an exception. It was established that genetic polymorphisms can influence the kinetics of the antineoplastic agent in the initial step of its activation. Di L (2019) has defined CES genes as highly polymorphic, based on the increasing number of reports of novel single-nucleotide polymorphisms affecting the therapeutic effect of drugs that are activated or eliminated through this biotransformation pathway (12). Literature analysis confirms that CES polymorphisms are an important predictor of the therapeutic effect and toxicity of CAP (13,17,33,34).

Impact of Age and Gender on CES Activity

Ontogenetic analyses show that the expression of CESs increases with the patient's age. In adults, the expression of CES1 and CES2 has been found to be ~ 50% and ~40% higher, respectively, than in children (35).

Reports on the impact of gender on CES activity are insufficient and contradictory. Zhu et al. (2009) are among the few researchers who have found that CES expression in humans is influenced by sex hormones but not the growth hormone (36). Cassidy J et al. (2019) have observed an 87% higher bioavailability of CAP in women than in men (37). Other researchers do not observe gender as a factor that should be considered in antineoplastic therapy (38).

Impact of Diseases on CES Activity

According to Yang et al. (2007) and Unver et al. (2018) high IL-6 levels lead to decreased expression of CES1 and CES2 (39, 40). Other studies demonstrate that type 2 diabetes mellitus, cardiovascular and hepatic diseases can also reduce CES levels (41, 42, 43). Therefore, in clinical practice, the presence of these diseases requires a dose adjustment of CAP, as well as individual monitoring of each patient (44).

CES Inhibitors and Inducers

As it was presented in Table 1, there is a large number of substrates of CES enzymes. The presence of some of them is followed by stimulation of transcription of CES genes, leading to increased enzyme expression (15). On the other hand, there are substances that can inactivate allosteric enzyme centres. Both of them (inducers and inhibitors) may affect the effectiveness of CAP therapy (45).

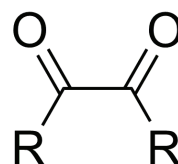
Inhibitors of Human CESs

Recombinant technologies have given the opportunity to screen hundreds of molecules and to distinguish those of them that showed activity only against human CESs (46). These molecules are usually classified as substances with a specific or a pan-inhibitory activity (47). Additionally, some of them inhibit reversibly or irreversibly the carboxylesterases' active center.

Reversible CES Inhibitors

1,2-diones - The number of in vitro studies concerning the pharmacotherapeutic and toxicological relevance of this type of CES inhibitors has increased in recent years. They are compounds that contain 1,2-dione functional group (Fig. 3) (48,49,50):

Their activity against CES enzymes exceeds the one toward acetylcholinesterase or butyrylcholinesterase enzymes (47). QSAR analyses show that, depending on the configuration of the 1,2-dione radical (cis- or trans-), some specificity is observed with respect to the CES1 or CES2 (51).



R = Alk, Ph,...

Fig. 3. 1,2 - dione structure

A number of authors have identified benzyl as a potent selective inhibitor of both carboxylesterases (52, 53). Janice L. Hyatt et al. (2006) have found that this inhibitor reduces the cytotoxic effect of irinotecan, because it inhibits the conversion to the active metabolite SN-38 (54). Tanshinone compounds, isolated from *Salvia miltiorrhiza*, also exhibit potent inhibitory activity against CESs. For example, M. Jason Hatfield et al. (2018) have demonstrated the ability of these molecules to modulate negatively the metabolism of oseltamivir (55).

Benzenesulfonamides

Some benzenesulfonate compounds are known to exhibit CES2-inhibitory activity (Fig. 4) (56, 57). By analogy with 1,2-dione, they also have no activity against acetylcholinesterases or butyrylcholinesterases (58, 59). In this regard, Randy M. Wadkins et al. (2004) have reported a suppression of gastrointestinal toxicity by irinotecan using benzenesulfonamide analogues. The authors have proved that the results are due to a selective inhibition of intestinal CES2 (56).

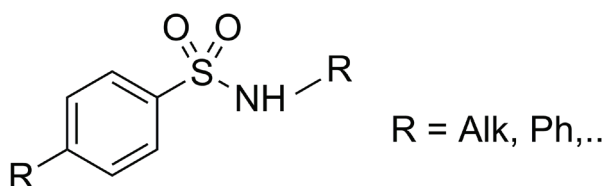


Fig. 4. Benzenesulfonamide structure

Trifluoroketones

Trifluoroketones exhibit strong inhibitory effects on carboxylesterases with K_i values in the low nanomolar range (Fig. 5) (60). Some members of this class have activity against other enzymes in the human body (61, 62). This should be taken into account when a selective drug-metabolizing modulation is intended.



Fig. 5. Trifluoroketone structure

Acylglucuronides

The number of scientific reports describing acylglucuronide metabolites as enzyme modulators (including toward CESs) is increasing (Fig. 6) (63, 64). Thereby, Williams et al. (2013) have reported that diclofenac- β -D-glucuronide and clopidogrel- β -D-glucuronide inhibit CES1-mediated hydrolysis of 4-nitrophenyl acetate (65).

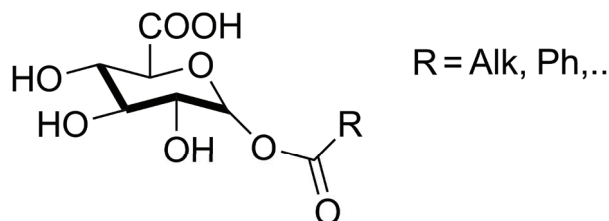


Fig. 6. Acylglucuronide structure

Terpenoids

Zou LW et al. (2017) have found that the pentacyclic triterpenoids oleanolic acid and ursolic acid have a strong inhibitory effect against CES1. Whereas, the β -boswellic acid exerts strong inhibitory effects against CES2 (66). In another study, Zou LW et al. (2016) have demonstrated that glycyrrhetic acid (a biologically active substance in the roots and rhizomes of *glycyrrhiza*) is a potent CES2-inhibitor (67).

Flavonoids

Bavachinin, coryfolin, corylin, neobavaisoflavone, corylifol A and corylifolinin contained in *Fructus psoraleae* (*Psoralea corylifolia* L. - Fabaceae) are reported to exhibit inhibitory properties against CES1 and CES2 (68, 69).

Another example of flavonoid-induced CES modulation is the ethanolic bark extract from white mulberry roots. It has been established that compounds contained therein (sanggenone D, kuwanon G and sanggenone C) inhibit CES2-mediated hydrolysis of fluorescein diacetate (70).

Fatty acid and cholesterol analogues

Crow et al. (2010) have reported that some natural fatty acids (myristic acid, myristolic acid, palmitic acid, palmitolic acid, linoleic acid, γ -linoleic acid, arachidonic acid) can strongly inhibit the hydrolytic activity of recombinant CES1. The authors have proven that 27-hydroxycholesterol also shows

inhibitory activity against recombinant CES1 and CES2 (71,72).

Medicines and excipients

The ability of CESs to biotransform a large variety of substances underlies the interactions between particular CES substrates, including drugs and/or excipients. Fukami et al. (2010) have reported that hydrolysis of imidapril (of recombinant CES1) is significantly inhibited by statins containing a lactone ring (simvastatin, lovastatin), as well as thiazolidinediones (troglitazone, rosiglitazone). Similarly, CES2-mediated hydrolysis of irinotecan can be strongly inhibited by both fenofibrate and simvastatin (73). In another in vitro study, Xu Yanjiao et al. (2013) have demonstrated that the antihypertensive drugs telmisartan and nitrendipine inhibit CES1-mediated hydrolysis of imidapril, and diltiazem and verapamil suppress CES2-hydrolysis of irinotecan (74). The anticholinesterase alkaloid physostigmine suppresses, by an identical mechanism, the decomposition of the mentioned above anticancer drug (75). The antidiarrheal medicine loperamide also demonstrates inhibitory activity against CES2 (76).

The literature review confirms the importance of excipients as potential participants in pharmacokinetic interactions too. Zhang et al. (2014) have reported that sodium lauryl sulphate and polyoxyl 40 hydrogenated castor oil can significantly inhibit imidapril's CES1-mediated hydrolysis. In addition, the non-ionic surfactant tween 20 and polyoxyl 35 castor oil can inhibit irinotecan's CES2-mediated hydrolysis (77).

Irreversible CES inhibitors

Carbamate compounds, which are widely used as insecticides, have been identified as potent inhibitors of acetylcholinesterases (78). Lyudmila G. Tsurkan et al. (2013) have proved their ability to inhibit CES1 and CES2 activity too (79). Organophosphates are another example of acetylcholinesterase and carboxylesterase inhibitors (80).

Inductors of Human CESs

The analysis of the literature data shows that there is a small number of studies dedicated to CES induction. Xu J et al. (2014) have asserted that glucose can stimulate hepatic CES1 expression in mice (81). Some antioxidants, such as sulforaphane compounds, are found to induce the same isozyme

(82,83). Medicines with CES-induction properties are dexamethasone and phenobarbital (84).

Possibilities for CES Therapeutic Modulation in CAP Treatment

Enzyme inhibitors/inductors are the only biotransformation factors that could be controlled and used in order to improve the pharmacological profile of a particular drug (85). Examples of successful fixed-dose combinations in modern practice are carbidopa/levodopa, amoxiclav/clavulanic acid, imipenem/cilastatin and others (86). Similarly, the administration of CES modulators in combination with CAP could be used for a prevention of gastrointestinal toxicity in cancer patients.

In this regard, Quinney SK et al. (2005) have reported that loperamide may exert its anti-diarrheal properties in CAP-induced GIT toxicity by an additional mechanism (76). Authors have proven that the opioid agonist is also a potent CES2 inhibitor. As a result, the amount of prematurely activated CAP (and respectively the formed cytotoxic agent 5-FU) in the gut was reduced. Accounting the pharmacokinetics of loperamide, an affection of CES1 activity is not expected (87). Moreover, the inhibition of CAP hydrolysis at CES2 level probably would lead to an improvement in its bioavailability. Therefore, modulation of CES activity could be used for a therapeutic process optimization, according to the individual patient.

CONCLUSION

There are several factors established that could be responsible for the deviation in activity and expression of CESs. In fact, they may influence the pharmacokinetics and efficacy of their drug substrates, such as CAP, which should be taken into account during the process of cancer treatment. On the other hand, the literature review showed that the initial step of CAP activation could be modulated as a therapeutic strategy. It was noted that the simultaneous intake of CAP and a selective CES2 inhibitor may be used in order to reduce the pro-drug GIT toxicity. Therefore, the encouraging results of these findings need further in vivo studies to reveal the full potential of the idea of CES modulation for therapeutic purposes.

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