Toxicology Letters 232 (2015) 159–166

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Contribution of human esterases to the metabolism of selected drugs of abuse

Markus R. Meyer *, Aline Schütz, Hans H. Maurer

Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, D-66421 Homburg (Saar), Germany

HIGHLIGHTS

- Characterization of the enzyme-catalyzed ester hydrolysis of ten drugs including eight drugs of abuse.
- Investigation of different esterase sources and isoforms.
- Kinetic parameters (V_{max}/K_m) determined for each reaction.
- Presented data important for prediction of drug–drug or drug–food interactions as well as for individual variations in metabolism.

ARTICLE INFO

Article history:
Received 29 September 2014
Received in revised form 18 October 2014
Accepted 21 October 2014
Available online 24 October 2014

Keywords:
Esterases
Drugs of abuse
Enzyme kinetics
Metabolism

ABSTRACT

Human esterases such as the human carboxylesterases (hCES) are important for the catalytic ester hydrolysis of xenobiotics and they play an important role in the detoxification of drugs (e.g., cocaine) but also in the activation of prodrugs (e.g., ramipril). Therefore, the aim of the presented study was to characterize the enzyme-catalyzed ester hydrolysis of ten drugs (cocaine, dimethocaine, ethylphenidate, 4-fluoro-3α-tropacocaine, 4-fluoro-3β-tropacocaine, heroin, methylphenidate, mitragynine, ramipril, and thebacon) by different esterase-containing systems (recombinant hCES1b, hCES1c, and hCES2, pooled human liver microsomes, pooled human liver S9 fraction, and pooled human plasma). Michaelis–Menten kinetic studies were done using in vitro incubations with the aforementioned enzyme-containing systems and LC coupled to ion trap MS for analysis. Ramipril and heroin were used as known model substrates to ensure reliable incubation conditions. The hydrolysis reactions followed classic Michaelis–Menten kinetics with exception of cocaine and 4-fluoro-3α-tropacocaine, for which hydrolysis rate was too low for reliable modeling. The substrates were mainly metabolized by the following enzymes systems: cocaine, hCES1c; dimethocaine, human plasma esterases; ethylphenidate, hCES1c; 4-fluoro-3β-tropacocaine, human plasma esterases; heroin, hCES2; methylphenidate, hCES1c; mitragynine, hCES1c; ramipril, hCES1b; thebacon, hCES2. Compounds bearing a small alcohol part and a larger acyl part showed higher affinity to hCES1 while those with a large alcohol part showed higher affinity to hCES2. The collected data are important for prediction of drug–drug or drug–food interactions as well as for individual variations in metabolism of drugs of abuse due to enzyme polymorphisms.

1. Introduction

Human carboxylesterases (hCES) are phase I drug-metabolizing enzymes and members of the serine hydrolase superfamily (Wang et al., 2011). CES are catalyzing the hydrolysis of several ester-containing therapeutic agents, drugs of abuse, and endogenous compounds (Thomsen et al., 2014; Wang et al., 2011) but can also activate prodrugs such as dabigatran etexilate (Laizure et al., 2014) or angiotensin-converting enzyme (ACE) inhibitors (Thomsen et al., 2014). These enzymes are responsible for the detoxification of numerous xenobiotics including heroin, cocaine, and methylphenidate (Hatfield et al., 2010). However, also the toxification of cocaine via transesterification to cocaethylene is catalyzed by hCES, in particular via the isoform hCES1 (Redinbo et al., 2003). Human CESs are a multigene family, in which isozymes are classified into five main groups (CES1–5) and several

* Corresponding author. Tel.: +49 6841 16 26430; fax: +49 6841 16 26051.
E-mail address: markus.meyer@uks.eu (M.R. Meyer).

http://dx.doi.org/10.1016/j.toxlet.2014.10.026
0378-4274/

subgroups according to the homology of the amino acid sequence (Laizure et al., 2013; Shimizu et al., 2014). The majority of hCESs belong to the hCES1 (two major isoforms hCES1b, and hCES1c) and hCES2 families (Brzezinski et al., 1997; Laizure et al., 2013; Pindel et al., 1997; Wang et al., 2011). They show differences in terms of tissue distribution, substrate specificity, and sequence identity (Wang et al., 2011). hCES in general is mainly localized in the endoplasmic reticulum of many tissues (Shimizu et al., 2014). hCES1 (in particular hCES1b) is primarily expressed in human liver and hCES2 mainly in the gastrointestinal tract and at lower levels in the liver (Wang et al., 2011). Although hCES1 and hCES2 have overlapping substrates, there are differences in terms of the substrate specificity. hCES1 prefers substrates with large acyl moieties and small alcohol parts, whereas hCES2 favors substrates with large alcohol substituents (Imai et al., 2006). However, hCES1 shares 47% sequence identity with human intestinal carboxylesterase hCES2 (Redinbo et al., 2003) and hCES1b/hCES1c are their major isoforms. Sequence alignment indicated that there are a few point mutations comparing hCES1b with hCES1c. For example several amino acids near the N-terminus are different (Wang et al., 2011). Besides hCES1 and hCES2, there are numerous other esterases expressed in humans such as four non-specific esterases contained in human blood (Li et al., 2005). At present, the role of the different hCES isoforms in the metabolism of many ester-containing compounds, particularly drugs of abuse, is still unclear.

Therefore, the aims of the presented work were to study the role of esterases in the metabolism of ten different drugs (chemical structures depicted in Fig. 1) using in vitro incubations and liquid chromatography–ion trap mass spectrometry (LC–ITMS). For six of them, data on the role of esterases in their metabolism have not been published so far. Furthermore, the enzyme kinetics of these tested compounds should be modeled in order to compare the substrate specificity of the different human esterases.

2. Materials and methods

2.1. Chemicals and reagents

Pooled human liver microsomes (pHLMs), pooled human liver S9 (pHS9), recombinant human carboxylesterases hCES1b, hCES1c, and hCES2 (prepared from baculovirus-transfected insert cells) were obtained from Corning (Utrecht, The Netherlands). After delivery, the enzymes were thawed at 37 °C, aliquoted, snap-frozen in liquid nitrogen, and stored at −80 °C until use. Fresh, pooled human blood plasma samples were from a local blood bank and stored at −80 °C until use. Inactive hCES was prepared by denaturation using heat and organic solvent 4-fluoro-3α-tropacocaine and its 3β isomer were synthesized as described elsewhere (Kavanagh et al., 2012). Hydrochlorides of ethylphenidate and dimethocaine were from LGC Standards (Luckenwalde, Germany), cocaine hydrochloride from Merck

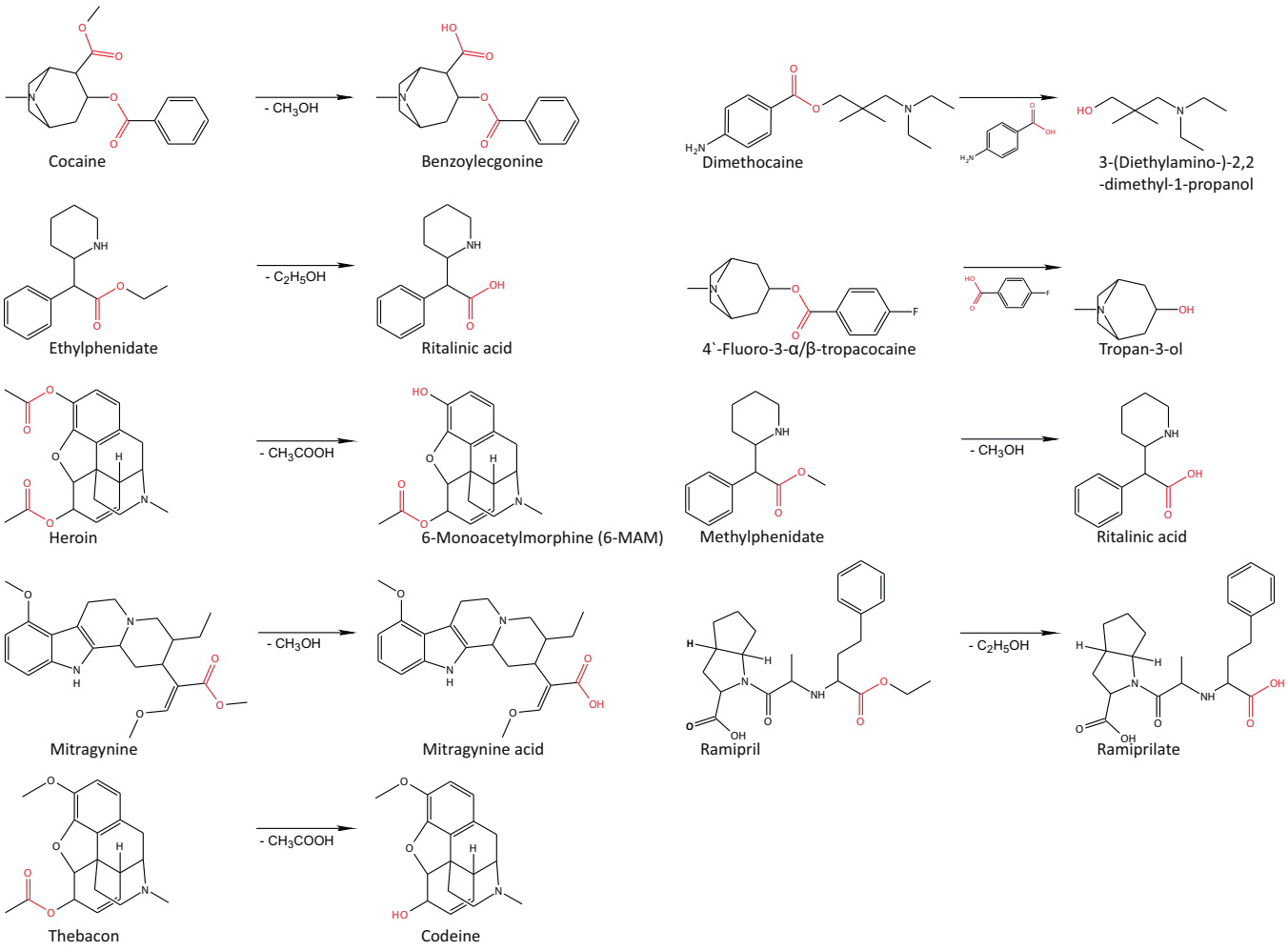

Fig. 1. Chemical structures of the studied compounds and their monitored hydrolysis products.