Toxicology in Vitro 25 (2011) 411–425

Contents lists available at ScienceDirect

Toxicology in Vitro

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

D-Isomer of *gly-tyr-pro-cys-pro-his-pro* peptide: A novel and sensitive *in vitro* trapping agent to detect reactive metabolites by electrospray mass spectrometry

Jaana E. Laine *, Seppo Auriola, Markku Pasanen, Risto O. Juvonen

University of Eastern Finland, Faculty of Health Sciences, School of Pharmacy, Yliopistonranta 1, P.O. Box 1627, FIN-70211 Kuopio, Finland

ARTICLE INFO

Article history:
Received 14 April 2010
Accepted 2 November 2010
Available online 12 November 2010

Keywords:
Bioactivation
LC/MS liquid chromatography mass spectrometry
Reactive metabolites
Covalent binding
Peptide D-isomer and peptide adducts

ABSTRACT

This paper describes a D-peptide isomer-based trapping assay using an LC/MS ion-trap spectrometer with an electrospray ionization (ESI) source as the analytical tool to study bioactivation of xenobiotics. Reactive metabolites were generated from parent compounds in *in vitro* incubations with different sources of CYP enzymes. A short D-isomer of *gly-tyr-pro-cys-pro-his-pro* proved to be a sensitive trapping agent and resistant to proteases. This method was tested with 16 probe substances. Acetaminophen, 1-chloro 2,4-dinitrobenzene, clozapine, diclofenac, imipramine, menthofuran, propranolol, pulegone and ticlopidine all formed D-peptide adducts, which were analogous to the GSH adducts previously described in the literature. New adducts were identified with clopidogrel (–Cl + peptide), nicotine ($-CH_3$+H + peptide), nimesulide (+peptide) and tolcapone (+peptide), i.e., no GSH adducts of those drugs have been described in the literature. No adducts were identified with ciprofloxacin, ketoconazole and verapamil. In the literature no GSH adducts have been described with ciprofloxacin and verapamil. D-Peptide-based trapping proved to be a reliable and reproducible method to identify bioactivated intermediates. D-Peptide is a new and convenient protein trapping agent for use in early phase screening of bioactivation of new chemical entities and evaluation of toxic properties of chemicals.

1. Introduction

The bioactivation of certain xenobiotics to reactive electrophiles is one of the key mechanisms of chemical induced toxicity, i.e., these compounds can physically bind to and react with proteins and/or nucleic acids. The adducts formed are thought to play critical roles both in short term toxicity and in the pathogenesis of chronic, degenerative diseases (Evans et al., 2004; Liebler and Guengerich, 2005; West and Marnett, 2006). The determination of the electrophiles and adducts is needed in the toxicological hazard and risk assessment. However, measuring the formation of electrophilic reactive metabolites is very demanding because of their instability and, furthermore, usually the amounts of these compounds produced are much smaller compared to major metabolites. Radioactive labelled compounds have long been used for kinetic studies and they are useful also for studying the bioactivation and adduction of xenobiotics to macromolecules but they require special synthesis and care in handling. Therefore, different types of trapping agents are used to measure the formation of the electrophiles. Glutathione (GSH) is the most commonly used trapping compound (Baillie and Davis, 1993; Inque et al., 2009; Ma and Surbanian, 2006; Masubushi et al., 2007; Rousu et al., 2009) since it forms conjugates with many kinds of electrophiles. Other trapping agents has also been used such as derivatives of glutathione *N*-acetylcysteine, γ-glutamylcysteinyllysine (Harada et al., 2009; Jian et al., 2009; Sieno et al., 2007a,b; Wen and Fitcg, 2009). Potassium cyanide (KCN) (Argoti et al., 2005; Evans et al., 2004; Gorrod and Aislaitner, 1994; Inque et al., 2009; Rousu et al., 2009), semicarbazide (Chen et al., 1997; Evans et al., 2004; Guan et al., 2008; Rousu et al., 2009; Zhang et al., 1996), methoxylamine (Yang and Chen, 2005) as well as a peptide γ-glutamylcysteteinyl lysine (Yan et al., 2007) and 11 aminoacid containing synthetic peptide (Mitchell et al., 2008) have also been used.

New liquid chromatography–mass spectrometry (LC/MS) techniques are very powerful analytical tools for elucidating the metabolism of xenobiotics (Clarke et al., 2001; Kang et al., 2009; Liu and Hop, 2005; Ma et al., 2006; Masubushi et al., 2007; Prakash et al., 2007; Soglia et al., 2004, 2006; Turpeinen et al., 2009). Ion trap instruments with a rapid scanning speed allow the sensitive detection of metabolites and the acquisition of their mass spectra in a single LC/MS run. The triple quadrupole-linear iontrap technique has opened further more possibilities in metabolite screening (Hopfgartner et al., 2004; Shou et al., 2005).

In this study, we developed a new peptide trapping agent for the detection of reactive intermediates. Electrophiles were produced from acetaminophen and 15 other structurally different compounds (Fig. 1) in *in vitro* incubations using control mouse liver microsomes as the enzyme source. Adducts were analyzed by LC/MS ion-trap

* Corresponding author. Tel.: +358 44 3372611; fax: +358 17 162131.
E-mail addresses: jaana.laine@uef.fi (J.E. Laine), seppo.auriola@uef.fi (S. Auriola), markku.pasanen@uef.fi (M. Pasanen), risto.juvonen@uef.fi (R.O. Juvonen).

0887-2333/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tiv.2010.11.002

mass-spectrometer with an electrospray ionization (ESI) source. We tested trapping properties of three synthetic peptides with acetaminophen, L-isomer γ-*glu-tyr-pro-cys-pro-his-pro* and L- and D-isomers *gly-tyr-pro-cys-pro-his-pro* peptides. Since the D-isomer proved to have the best properties its trapping ability was also tested with 16 structurally different compounds (acetaminophen, 1-chloro 2,4-dinitrobenzene, ciprofloxacin, clopidogrel, clozapine, diclofenac, imipramine, ketoconazole, menthofuran, nicotine, nimesulide, propranolol, pulegone, ticlopidine, tolcapone and verapamil). The metabolism of these compounds is associated with the formation of different types of electrophiles (hard and soft electrophiles).

2. Materials and methods

2.1. Chemicals

Reduced glutathione (GSH), potassium phosphate, NADPH, $MgCl_2$, 1-chloro 2,4-dinitrobenzene, ciprofloxacin, clopidogrel,

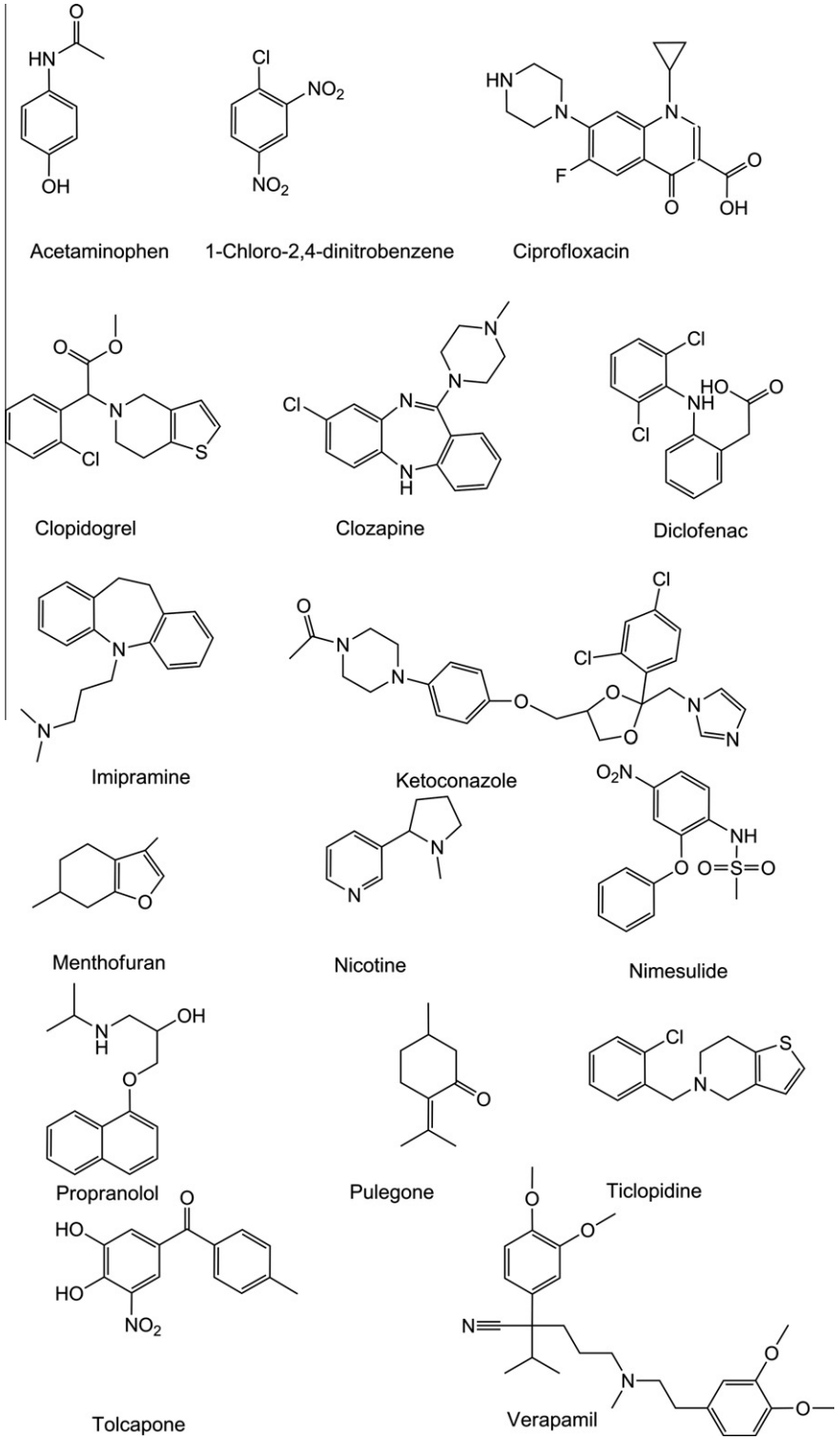

Fig. 1. The tested 16 compounds trapped with D-peptide isomer *gly-tyr-pro-cys-pro-his-pro* (peptide 3).