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A GC–MS/MS method for the quantitative analysis of low levels of the tyrosine metabolites maleylacetone, succinylacetone, and the tyrosine metabolism inhibitor dichloroacetate in biological fluids and tissues

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Abstract

We developed a sensitive method to quantitate the tyrosine metabolites maleylacetone (MA) and succinylacetone (SA) and the tyrosine metabolism inhibitor dichloroacetate (DCA) in biological specimens. Accumulation of these metabolites may be responsible for the toxicity observed when exposed to DCA. Detection limits of previous methods are 200 ng/mL (1.2 pmol/μL) (MA) and 2.6 μg/mL (16.5 pmol/μL) (SA) but the metabolites are likely present in lower levels in biological specimens. To increase sensitivity, analytes were extracted from liver, urine, plasma and cultured nerve cells before and after dosing with DCA, derivatized to their pentafluorobenzyl esters, and analyzed via GC–MS/MS.

Keywords: DCA; MA; SA; GC–ECNCI-MS; PFB-Br; Tyrosine metabolites

1. Introduction

Perturbation of human tyrosine catabolism (Fig. 1) may occur through several well-known inborn errors of metabolism or by xenobiotics [1]. Loss of function mutations in the terminal catabolic enzyme, fumarylacetoacetate hydrolase, cause hereditary tyrosinemia type 1 (HT1) and the accumulation of upstream intermediates, such as fumarylacetoacetate, fumarylacetone (FA), maleylacetoacetate and maleylacetone (MA). The acetate derivatives have not been directly measured in biological tissues or fluids from affected patients and, to date, only the urinary accumulation of MA has been reported in HT1. Nevertheless, these reactive molecules are thought to be responsible for the high incidence of hepatocellular carcinoma in children with HT1 [1]. Inhibition of fumarylacetoacetate hydolase also causes diversion of tyrosine intermediates to succinylacetone (SA), the urinary accumulation of which is pathonomonic of HT1 [2,3]. In turn, SA inhibits a proximal step in heme synthesis, causing accumulation of delta-aminolevulinate. These biochemical consequences of SA are thought to account for the neuropathic complications of HT1, including peripheral neuropathy [1].

The penultimate enzyme of tyrosine catabolism, maleylacetoacetate isomerase (MAAI), catalyzes the isomerization of maleylacetoacetate to fumarylacetoacetate and of MA to FA. MAAI is identical to the zeta1 family isoform of glutathione *S*-transferase (GSTz1), which dehalogenates a number of short chain haloacids, including dichloroacetate (DCA). This xenobiotic is unusual in that it is present ubiquitously in the biosphere, in part because of its environmental contamination as a disinfection by-product of water chlorination and as a metabolite of industrial degreasing agents, such as the Superfund chemical trichloroethylene [4]. Although typical daily human exposure to DCA is probably in the low μg/kg body weight range or less, the compound is also used as an investigational drug for certain acquired and congenital metabolic diseases, in which daily oral or intravenous administration approximates 10–50 mg/kg or more [5].

Over the so-called "environmental" (μg/kg per day)—"clinical" (mg/kg/d) concentration rage, DCA inhibits its own

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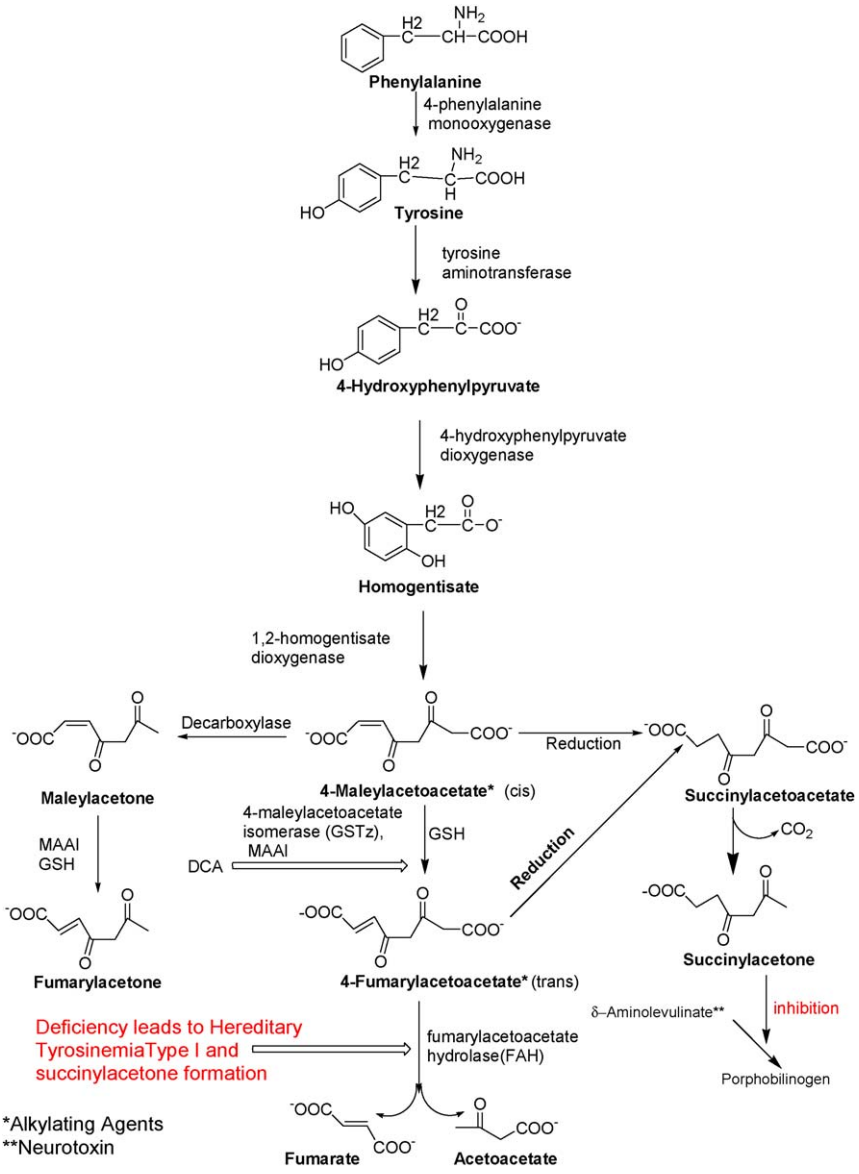

Fig. 1. Tyrosine catabolic pathway.

metabolism, apparently by inhibiting the activity and decreasing the expression of MAAI/GSTz1 [4,6,7]. This has been demonstrated in humans [8] and rats, whereby repeated DCA exposure is associated with a decrease in its plasma clearance and an increase in its plasma half life [9]. In rats, hepatic activity and expression of MAAI/GSTz1 are reversibly decreased in a dose- and duration-dependent manner [9,10].

Current analytical techniques provide detection limits of 200 mg/mL (1.2 pmol/μL) for MA and 2.5 μg/mL (16.5 pmol/μL) for SA [11]. However, the levels of these metabolites are probably present in much lower concentrations in blood and tissues. Indeed, previous methods have not detected SA in plasma. Although DCA is readily measured in blood and urine by current techniques, [11,12] its quantitation in cells has been problematic without prior isotopic labeling of the molecule [13]. Thus, improvement in the ability to detect low concentrations of MA, SA and DCA could have broad application to the study of normal amino acid metabolism and the effects of genetic or pharmacological perturbations thereon.

Electron capture is a highly sensitive ionization technique and can be used to improve the detection limits of electron ionization (EI) methods [14–16]. The use of a derivatizing agent with high electron capture ability and the capacity to provide stable negative ions results in increased sensitivity over positive CI or EI. A common derivatizing agent for this procedure is pentafluorobenzyl (PFB) bromide [15,16]. In the ionization source electron capture negative chemical ionization (ECNCI), gas phase PFB-analytes are bombarded with thermal electrons produced from high energy collisions of electrons with the CI gas. The five highly electronegative fluoro atoms of PFB derivatives are efficient in capturing thermal electrons. The reaction with the thermal electrons results in fragmentation of the PFB-analyte at the ester bond forming the stable anion of the carboxylic acid. These ions are then focused and analyzed in the ion trap mass