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Inhibition of human alcohol and aldehyde dehydrogenases by acetaminophen: Assessment of the effects on first-pass metabolism of ethanol

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ABSTRACT

Acetaminophen is one of the most widely used over-the-counter analgesic, antipyretic medications. Use of acetaminophen and alcohol are commonly associated. Previous studies showed that acetaminophen might affect bioavailability of ethanol by inhibiting gastric alcohol dehydrogenase (ADH). However, potential inhibitions by acetaminophen of first-pass metabolism (FPM) of ethanol, catalyzed by the human ADH family and by relevant aldehyde dehydrogenase (ALDH) isozymes, remain undefined. ADH and ALDH both exhibit racially distinct allozymes and tissue-specific distribution of isozymes, and are principal enzymes responsible for ethanol metabolism in humans. In this study, we investigated acetaminophen inhibition of ethanol oxidation with recombinant human ADH1A, ADH1B1, ADH1B2, ADH1B3, ADH1C1, ADH1C2, ADH2, and ADH4, and inhibition of acetaldehyde oxidation with recombinant human ALDH1A1 and ALDH2. The investigations were done at near physiological pH 7.5 and with a cytoplasmic coenzyme concentration of 0.5 mM NAD^+. Acetaminophen acted as a noncompetitive inhibitor for ADH enzymes, with the slope inhibition constants (K_{is}) ranging from 0.90 mM (ADH2) to 20 mM (ADH1A), and the intercept inhibition constants (K_{ii}) ranging from 1.4 mM (ADH1C allozymes) to 19 mM (ADH1A). Acetaminophen exhibited noncompetitive inhibition for ALDH2 (K_{is} = 3.0 mM and K_{ii} = 2.2 mM), but competitive inhibition for ALDH1A1 (K_{is} = 0.96 mM). The metabolic interactions between acetaminophen and ethanol/acetaldehyde were assessed by computer simulation using inhibition equations and the determined kinetic constants. At therapeutic to subtoxic plasma levels of acetaminophen (i.e., 0.2–0.5 mM) and physiologically relevant concentrations of ethanol (10 mM) and acetaldehyde (10 μM) in target tissues, acetaminophen could inhibit ADH1C allozymes (12–26%) and ADH2 (14–28%) in the liver and small intestine, ADH4 (15–31%) in the stomach, and ALDH1A1 (16–33%) and ALDH2 (8.3–19%) in all 3 tissues. The results suggest that inhibition by acetaminophen of hepatic and gastrointestinal FPM of ethanol through ADH and ALDH pathways might become significant at higher, subtoxic levels of acetaminophen.

Introduction

Acetaminophen (paracetamol; *N*-acetyl-*p*-aminophenol) is a common analgesic and antipyretic drug, one of the most widely used over-the-counter medications in the world. It has been well documented that chronic excessive alcohol exposure enhances hepatotoxicity of acetaminophen by elevation of ethanol-inducible cytochrome P450 2E1 (CYP2E1), which converts acetaminophen into reactive toxic intermediates. In contrast, simultaneous alcohol exposure may exert a protective effect due to inhibition by ethanol of the CYP2E1 biotransformation of acetaminophen (Cederbaum, 2012; Lieber, 2004; Riordan & Williams, 2002). However, the interaction of acetaminophen with ethanol metabolism has received much less attention. Acetaminophen was reported to inhibit gastric alcohol dehydrogenase (ADH) activity (Palmer, Frank, Nambi, Wetherington, & Fox, 1991; Roine, Rosman, Gentry, Baraona, & Lieber, 1991). Acetaminophen's potential inhibition of other ADH family members and aldehyde dehydrogenase (ALDH) isozymes in relation to ethanol metabolism remains unknown. Use of acetaminophen and alcohol are commonly associated. Acetaminophen may increase blood alcohol levels *in vivo*, particularly at a low alcohol dose, thus having potential clinical consequences as

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well as influencing the performance of drivers who have consumed alcohol (Jones, 2010; Lieber, Gentry, & Baraona, 1996).

First-pass, or presystemic, metabolism (FPM) of ethanol affects the peripheral availability of ethanol, thus influencing ethanol's intoxicating effects in the body. The sites of FPM include the stomach, small intestine, and liver, but their relative contributions to ethanol metabolism remain controversial (Badger et al., 2003; Gentry, Baraona, & Lieber, 1994; Levitt, 1994; Yin, Lee, Yao, & Lai, 2007). Several factors may affect the extent of the FPM of ethanol, such as food consumption, concentration of alcoholic beverages, genetic polymorphism of alcohol-metabolizing enzymes, and medications that interfere with activity of the metabolizing enzymes or with absorption of ethanol (Cederbaum, 2012; Jones, 2010; Kalant, 1996; Lee, Chau, Yao, Wu, & Yin, 2006).

ADH and ALDH catalyze oxidation of various aliphatic/aromatic endogenous and exogenous alcohols to their corresponding aldehydes, and then to their corresponding carboxylic acids, respectively (Edenberg & Bosron, 2010; Höög, Stromberg, Hedberg, & Griffiths, 2003; Sophos & Vasiliou, 2003; Wang, Han, & Yin, 2009). Both ADH and ALDH, the principal enzymes responsible for metabolism of ethanol in humans (Cederbaum, 2012; Yin & Agarwal, 2001), exhibit functional polymorphisms among racial populations and tissue-specific distributions. Human ADH family members have been categorized into 5 classes based on 1) protein sequence, 2) gene organization, 3) electrophoretic features, 4) kinetic features, and 5) immunochemical features (Duester et al., 1999; Höög & Ostberg, 2011; Lee, Chiang, et al., 2006). Class I ADH contains multiple forms, i.e., ADH1A (previously denoted αα), ADH1B (ββ), and ADH1C (γγ). ADH classes II–IV contain a single form each, i.e., ADH2 (ππ), ADH3 (χχ), and ADH4 (μμ or σσ), respectively. *ADH1B*1* (encoding the β_1 polypeptide subunit) and *ADH1B*2* (encoding the β_2 subunit) are predominant among Caucasians and East Asians, respectively. *ADH1B*3* (encoding the β_3 subunit) is found exclusively in Africans and some tribes of American Indians. *ADH1C*1* (encoding the γ_1 subunit) and *ADH1C*2* (encoding the γ_2 subunit) are approximately equally distributed among Caucasians and American Indians, but *ADH1C*1* is highly prevalent among East Asian and African populations. Currently, class V ADH is the only family member with no available data for catalytic function due to its extremely labile activity (Höög et al., 2003; Ostberg, Stromberg, Hedberg, Persson, & Höög, 2013). All 3 class I isozymes, ADH2, and ADH3 are expressed in human liver (Edenberg & Bosron, 2010; Yin & Agarwal, 2001), while ADH4 and ADH1C are detected in the stomach (Yin et al., 1997), and ADH2 and ADH1C are detected in the small intestine (Chiang, Wu, et al., 2012).

In the human ALDH superfamily (Anonymous, 1989; Sládek, 2003; Weiner & Ho, 2007), class I ALDH1A1 and class II ALDH2 are predominantly expressed in human liver (Yao, Liao, & Yin, 1997), and both isozymes are detected in the gastrointestinal tract (Chiang, Jao, et al., 2012; Chiang, Wu, et al., 2012; Yin et al., 1997), whereas class III ALDH3A1 is a major form found in the stomach (Yin et al., 1997). Mitochondrial ALDH2 is the major isozyme for oxidation of acetaldehyde *in vivo* due to its submicromolar K_m and high catalytic efficiency, whereas cytosolic ALDH1A1 (with its high micromolar K_m) may also contribute to oxidation of acetaldehyde, particularly for individuals who lack active ALDH2 (Peng & Yin, 2009; Yin & Peng, 2005). About 40% of East Asians are deficient in ALDH2 activity due to the dominant negative variant allele of *ALDH2*2* (Crabb, Matsumoto, Chang, & You, 2004; Lai et al., in press). This deficiency has been attributed to protection against development of alcoholism (Chen, Peng, Tsao, et al., 2009; Chen, Peng, Wang, Tsao, & Yin, 2009), but it is a risk factor for alcohol-related diseases such as esophageal cancer (Brooks, Enoch, Goldman, Li, & Yokoyama, 2009; Yin & Agarwal, 2001).

To investigate potential metabolic interactions between ethanol and acetaminophen, we describe, from an enzymological and pharmacogenetic perspective, the inhibition of human ADH isozymes/allozymes and relevant ALDH isozymes at a physiological concentration of coenzyme NAD^+. We also describe the simulation of effects of ethanol and acetaldehyde, at physiological levels.

Materials and methods

Expression and purification of human ADH and ALDH

As described previously (Chiang et al., 2009; Lee, Chau, et al., 2006; Lee, Chiang, et al., 2006), *Escherichia coli* was used to express recombinant human enzymes. The following human enzymes were then purified to the apparent homogeneity: ADH1A, ADH1B1, ADH1B2, ADH1B3, ADH1C1, ADH1C2, ADH2, ADH3, ADH4; and ALDH1A1, ALDH2, ALDH3A1. All of the isolated recombinant enzyme forms exhibited a single coomassie blue-staining protein band with molecular masses of 40 kDa, 55 kDa, and 54 kDa for ADHs, ALDH1A1/2, and ALDH3A1, respectively, on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Protein concentration was determined by the method of Lowry, Rosebrough, Farr and Randall (1951) using bovine serum albumin as the standard.

Kinetic analysis

Kinetic studies for ADH and ALDH were performed in 0.1 M sodium phosphate at pH 7.5 and 25 °C, containing 0.5 mM NAD^+, 1 mM ethylenediaminetetraacetate (EDTA; only for ALDH assay), and varied concentrations of substrate and inhibitor. The cytosolic NAD^+ concentration in rat hepatocytes is reported to be approximately 0.5 mM (Bücher et al., 1972). The enzyme activity was determined by monitoring the production of NADH at 340 nm using an absorption coefficient of 6.22 mM^{-1} cm^{-1} for the ADH and ALDH assays (unless otherwise indicated), or at 460 nm for emission of the fluorescence for the assays of ADH3, ALDH1A1, and ALDH2. Preparation of formaldehyde and calculation of the concentration of *S*-hydroxymethylglutathione from the equilibrium constants were performed as described previously (Lee, Wang, Lee, & Yin, 2003). Acetaldehyde and benzaldehyde were redistilled before use. The reaction was initiated with addition of the enzyme. Enzyme activity units (U) are expressed as micromoles of NADH formed per minute. Steady-state kinetic data were analyzed by nonlinear least-squares regression using the Cleland programs of HYPER, COMP, NONCOMP, and UNCOMP (Cleland, 1979). Initial velocity data were fitted with the HYPER program to the Michaelis–Menten equation.

$$v = (V_{max} \times S)/(K_m + S) \quad (1)$$

The data from dead-end inhibition studies were fitted with the following linear inhibition equations, i.e., the COMP program for competitive inhibition, the NONCOMP for noncompetitive inhibition, and the UNCOMP for uncompetitive inhibition, respectively.

$$v = (V_{max} \times S)/[K_m(1 + I/K_{is}) + S] \quad (2)$$

$$v = (V_{max} \times S)/[K_m\,(1 + I/K_{is}) + S\,(1 + I/K_{ii})] \quad (3)$$

$$v = (V_{max} \times S)/[K_m + S\,(1 + I/K_{ii})] \quad (4)$$

where V_{max} is the maximum velocity, S is the substrate concentration, K_m is the Michaelis constant, I is the inhibitor concentration, and K_{is} and K_{ii} are the slope and intercept inhibition constants,