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Ethanol oxidation and the inhibition by drugs in human liver, stomach and small intestine: Quantitative assessment with numerical organ modeling of alcohol dehydrogenase isozymes

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ABSTRACT

Alcohol dehydrogenase (ADH) is the principal enzyme responsible for metabolism of ethanol. Human ADH constitutes a complex isozyme family with striking variations in kinetic function and tissue distribution. Liver and gastrointestinal tract are the major sites for first-pass metabolism (FPM). Their relative contributions to alcohol FPM and degrees of the inhibitions by aspirin and its metabolite salicylate, acetaminophen and cimetidine remain controversial. To address this issue, mathematical organ modeling of ethanol-oxidizing activities in target tissues and that of the ethanol–drug interactions were constructed by linear combination of the corresponding numerical rate equations of tissue constituent ADH isozymes with the documented isozyme protein contents, kinetic parameters for ethanol oxidation and the drug inhibitions of ADH isozymes/allozymes that were determined in 0.1 M sodium phosphate at pH 7.5 and 25 °C containing 0.5 mM NAD^+. The organ simulations reveal that the ADH activities in mucosae of the stomach, duodenum and jejunum with *ADH1C*1/*1* genotype are less than 1%, respectively, that of the *ADH1B*1/*1–ADH1C*1/*1* liver at 1–200 mM ethanol, indicating that liver is major site of the FPM. The apparent hepatic K_M and V_{max} for ethanol oxidation are simulated to be 0.093 ± 0.019 mM and 4.0 ± 0.1 mmol/min, respectively. At 95% clearance in liver, the logarithmic average sinusoidal ethanol concentration is determined to be 0.80 mM in accordance with the flow-limited gradient perfusion model. The organ simulations indicate that higher therapeutic acetaminophen (0.5 mM) inhibits 16% of *ADH1B*1/*1* hepatic ADH activity at 2–20 mM ethanol and that therapeutic salicylate (1.5 mM) inhibits 30–31% of the *ADH1B*2/*2* activity, suggesting potential significant inhibitions of ethanol FPM in these allelotypes. The result provides systematic evaluations and predictions by computer simulation on potential ethanol FPM in target tissues and hepatic ethanol–drug interactions in the context of tissue ADH isozymes.

1. Introduction

Alcohol dehydrogenase (ADH) is the principal enzyme responsible for metabolism of ethanol in humans [1–3]. Cytochrome P450 CYP2E1, which is induced by alcohol, plays a secondary role in removal of ingested alcohol, especially at high concentrations [1,2]. The ADH constitutes a complex isozyme family with broad substrate specificity, catalyzing oxidation of a wide variety of aliphatic and aromatic alcohols to the corresponding aldehydes [4]. On the basis of protein sequence, genomic organization, electrophoretic mobility, kinetic property and immunochemical features, human ADH family has been categorized into five classes [5–7]. The class I ADH contains multiple isozymes: ADH1A (previously denoted αα),

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ADH1B (ββ), and ADH1C (γγ). Class II to IV ADHs contain a single isozyme each, that is, ADH2 (ππ), ADH3 (χχ), and ADH4 (μμ or σσ), respectively. ADH1B and ADH1C exhibit allozymes arising from allelic variations of the corresponding genes [4,8]. *ADH1B*1* (encoding the β_1 subunit polypeptide) and *ADH1B*2* (encoding β_2 subunit) are predominant among Caucasians and East Asians. *ADH1B*3* (encoding β_3 subunit) is found exclusively in Africans and some tribes of American Indians. *ADH1C*1* (encoding γ_1 subunit) and *ADH1C*2* (encoding γ_2 subunit) are approximately equally distributed among Caucasians and American Indians, but the former is highly prevalent among the East Asian and African populations. Currently, class V ADH is the only isozyme unavailable for catalytic function due to its extreme instability [9]. Human ADH family also exhibits tissue-specific distribution: all three Class I isozymes and ADH2 are detected in the liver [10], ADH4 and ADH1C in the gastric mucosa [11], ADH2 and ADH1C in the mucosae of small intestine [12], whereas ADH3 appears ubiquitously [4].

The liver functions as chief organ for human ethanol elimination [10]. The stomach and small intestine are responsible for absorption of ingested alcohol [13]. Both liver and gastrointestinal tract are potential sites for first-pass metabolism (FPM) of ingested ethanol that influences the bioavailability and intoxicating effects of alcohol [14,15]. Several factors affect the ethanol FPM, such as concomitant intake of food, concentration of alcoholic beverages, and medications that inhibit ADH activity in the target tissues or perturb gastric emptying [13–15]. Aspirin and acetaminophen are analgesic, antipyretic agents, both widely used over-the-counter medications. Cimetidine, one of the H_2-receptor antagonists, is an over-the-counter medication for gastric and duodenal ulcers. Chronic use of these drugs and alcohol are commonly associated [16,17]. It has been documented that aspirin, acetaminophen and cimetidine increased blood ethanol concentrations by inhibiting FPM of ethanol [18–20] but degrees of the inhibition and hepatic or gastric origin of the drug actions remain controversial [13,14,21,22]. Recently, the inhibition kinetics of ethanol oxidation with human ADH isozymes/allozymes by aspirin and its major metabolite salicylate [23], acetaminophen [24], and cimetidine [25] has been reported.

To assess and predict potential capacity of ethanol FPM and degrees of inhibition by the above commonly used drugs at an organ level, it would need both the pertinent kinetic parameters and total protein contents of the constituent ADH isozymes in target tissues. We report here an attempt to address this issue by constructing organ simulation models with numerical rate equations of the component ADH isozymes in human liver and gastrointestinal mucosae. To answer such a complex question involving multiple functional genetic polymorphisms, the computer simulations focused on an individual with representative combinatorial genotype of *ADH1B*1/*1* and *ADH1C*1/*1*, both the most prevalent allelic variants among world populations, for FPM of ethanol and the alcohol–drug interactions. In latter case, individuals with ADH1B1 and ADH1B2 allozymes were also compared.

2. Materials and methods

2.1. Recombinant human ADH isozymes

All kinetic data used in the present numerical modeling studies were obtained with purified recombinant ADHs. The expression and purification of human recombinant ADH isozymes were described previously [23–26]. Protein concentration of the purified isozymes was determined by the Lowry method [27] using bovine serum albumin as the standard.

2.2. Protein content of ADH isozymes in human tissues

The ADH isozyme protein contents in human liver and gastrointestinal mucosae are summarized in supplementary Table S1. The isozyme protein amount in normal portions of the surgical liver specimens and mucosae of the duodenum and jejunum were determined by immunoblot using the corresponding affinity-purified class-specific antibodies and the respective purified recombinant ADH isozymes as the standard [10,12]. All patients provided written informed consent and the studies were approved by the Institutional Review Board of the National Defense Medical Center [10–12]. It is also noted that sex and age did not significantly influence hepatic and gastrointestinal mucosal ADH activities with specified genetic phenotypes [10–12]. The ratio of the total subunit contents of class I ADH1A, ADH1B and ADH1C in liver was estimated from CM-cellulose chromatography of the isolated mixture of class I isozymes [26]. The protein content of gastric ADH isozymes was estimated from the isozyme activities in surgical gastric mucosae and the specific activity of the purified isozymes [11,26]. The liver mass for a 70-kg man was estimated as 2% body weight, that is, 1400 g [26]. The estimates of total mucosa mass of human stomach, duodenum, and jejunum were 23, 34, and 149 g, respectively [28].

2.3. Formulation of numerical organ models

2.3.1. Composite rate equations for FPM of ethanol

Unlike the vast majority of drugs and xenobiotics, the elimination of ethanol is not proportional to its concentration in body fluids, that is, first-order kinetics. Instead, elimination of ethanol exhibits pseudolinear, near zero-order kinetics at concentrations of blood ethanol higher than 3 mM [1,14,21]. This Michaelis–Menten type of pharmacokinetics is of special interest in FPM, which is defined as the presystemic elimination of newly absorbed ethanol through the mucosal tissue of stomach and small intestine, as well as the liver through portal vein, before reaching peripheral blood. Thus, saturating ADH isozymes with absorbed (or recirculating) ethanol would drastically reduce further FPM in the target tissues. Kinetic analysis of ethanol oxidation with the relevant constituent ADH isozymes in target tissues of FPM in the absence or presence of drug inhibitors was determined in 0.1 M sodium phosphate at pH 7.5 and 25 °C, containing 0.5 mM NAD^+ (a cytosolic concentration), and varied concentrations of substrate and inhibitor [23–26]. The resultant kinetic data were applied in construct of organ modelings.

Four basic steady-state kinetic equations were used in building the models, that is, Michaelis–Menten equations in the absence [Eq. (1)] and in the presence of competitive inhibitor [Eq. (2)], uncompetitive inhibitor [Eq. (3)], or noncompetitive inhibitor [Eq. (4)] as following:

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

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

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

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

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

In the flow-limited, concentration-gradient sinusoidal perfusion model of hepatic drug metabolism [29,30], the rate (v) of drug