Biochemical Pharmacology 95 (2015) 71–79

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

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

Shou-Lun Lee [a,*], Yung-Pin Lee [b], Min-Li Wu [b], Yu-Chou Chi [b], Chiu-Ming Liu [b], Ching-Long Lai [c], Shih-Jiun Yin [b]

[a] Department of Biological Science and Technology, China Medical University, 91 Hsueh-Shih Road, Taichung 40402, Taiwan
[b] Department of Biochemistry, National Defense Medical Center, 161 Minchuan East Road Section 6, Taipei 11453, Taiwan
[c] Department of Nursing, Chang Gung University of Science and Technology, 261 Wenhwa 1st Road, Kweishan Township, Taoyuan 33303, Taiwan

ARTICLE INFO

Article history:
Received 19 January 2015
Accepted 5 March 2015
Available online 13 March 2015

Keywords:
Aspirin
Salicylate
Alcohol dehydrogenase (ADH)
Aldehyde dehydrogenase (ALDH)
Inhibition kinetics
Alcohol first-pass metabolism

ABSTRACT

Previous studies have reported that aspirin significantly reduced the first-pass metabolism (FPM) of ethanol in humans thereby increasing adverse effects of alcohol. The underlying causes, however, remain poorly understood. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), principal enzymes responsible for metabolism of ethanol, are complex enzyme families that exhibit functional polymorphisms among ethnic groups and distinct tissue distributions. We investigated the inhibition profiles by aspirin and its major metabolite salicylate of ethanol oxidation by recombinant human ADH1A, ADH1B1, ADH1B2, ADH1B3, ADH1C1, ADH1C2, ADH2, and ADH4, and acetaldehyde oxidation by ALDH1A1 and ALDH2, at pH 7.5 and 0.5 mM NAD^+. Competitive inhibition pattern was found to be a predominant type among the ADHs and ALDHs studied, although noncompetitive and uncompetitive inhibitions were also detected in a few cases. The inhibition constants of salicylate for the ADHs and ALDHs were considerably lower than that of aspirin with the exception of ADH1A that can be ascribed to a substitution of Ala-93 at the bottom of substrate pocket as revealed by molecular docking experiments. Kinetic inhibition equation-based simulations show at higher therapeutic levels of blood plasma salicylate (1.5 mM) that the decrease of activities at 2–10 mM ethanol for ADH1A/ADH2 and ADH1B2/ADH1B3 are predicted to be 75–86% and 31–52%, respectively, and that the activity decline for ALDH1A1 and ALDH2 at 10–50 μM acetaldehyde to be 62–73%. Our findings suggest that salicylate may substantially inhibit hepatic FPM of alcohol at both the ADH and ALDH steps when concurrent intaking aspirin.

1. Introduction

Aspirin is one of the most widely prescribed analgesic, antipyretic and antiinflammatory agents. Because the drug is so generally available, use of aspirin and alcoholic beverages is commonly associated [1,2]. It has been shown that aspirin increases blood ethanol concentrations by inhibition of first-pass metabolism (FPM) of ethanol [3–5], thereby increasing the adverse effects of alcohol such as impaired driving. The major sites of ethanol FPM include the stomach, small intestine, and liver, but their relative contributions to alcohol metabolism remain controversial [3,6–8]. Several factors may affect 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 alcohol [3,6,9].

Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are principal enzymes responsible for metabolism of ethanol in humans, catalyzing conversion of ethanol to acetaldehyde and then to acetate, respectively [8,9]. Both enzymes exhibit functional polymorphisms among racial populations and tissue-specific distributions [10–12]. Human ADH family members have been categorized into five classes on the basis of protein sequence, genetic organization, electrophoretic mobility, kinetic property, and immunochemical features [13–15]. The class I ADH contains multiple forms: ADH1A (previously denoted αα), ADH1B (ββ), and ADH1C (γγ). ADH classes II to IV each contain a single form: ADH2 (ππ), ADH3 (χχ), and ADH4 (μμ or σσ). ADH1B and ADH1C

* Corresponding author. Tel.: +886 4 22053366x2526; fax: +886 4 22051507.
E-mail address: sllee@mail.cmu.edu.tw (S.-L. Lee).

http://dx.doi.org/10.1016/j.bcp.2015.03.003
0006-2952/

exhibit allelic variations [11,12]. *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 family member unavailable for catalytic function due to its extreme instability [16]. All three class I isozymes, ADH2 and ADH3 are expressed in human adult liver [12,17], while ADH4 and ADH1C are detected in the stomach [18], and ADH2 and ADH1C are detected in the small intestine [19].

In the human ALDH superfamily [20,21], class I ALDH1A1 and class II ALDH2 are predominantly expressed in human liver [12] and both isozymes are detected in the gastrointestinal tract [18,19], whereas class III ALDH3A1 is a major form found in the stomach [18]. Mitochondrial ALDH2 is the primary isozyme for oxidation of acetaldehyde derived from ethanol due to its submicromolar K_m and high catalytic efficiency, whereas cytoplasmic ALDH1A1 with micromolar K_m also contributes to oxidation of the acetaldehyde, particularly in individuals who lack active ALDH2 [12,22]. About 40% of East Asians are deficient in ALDH2 activity as a result of the dominant negative variant allele *ALDH2*2* [10,23]. This deficiency has been attributed to protection against development of alcoholism [11,24], but it is a risk factor for alcohol-related diseases such as esophageal cancer [25,26].

After oral administration, aspirin is rapidly broken down by liver, erythrocyte, and plasma esterases to salicylate [27,28]. Lieber and colleagues [4,5] have reported that aspirin (1 g taken with a standard breakfast) significantly increased alcohol bioavailability (at a test dose 0.3 g/kg of ethanol 1 h after the beginning of the meal) with a concomitant 39% decrease in the FMP of alcohol whereas it did not significantly influence the gastric emptying. However, there has been lacking a systematic investigation with regard to inhibitions by aspirin and salicylate of human ADHs and ALDHs in the context of FPM of ethanol. We report herein, the inhibition types/constants and the kinetic equation-based simulation of human ADH family members and the pertinent ALDH isozymes, at a near physiological coenzyme concentration and pH, under pharmacologically relevant concentration ranges of ethanol/acetaldehyde and the interacting drugs.

2. Methods

2.1. Chemicals

Aspirin, salicylic acid, sodium phosphate monobasic, acrylamide, *N,N,N′,N′*-tetramethylethylenediamine, hexamethylenetetramine, sodium dodecyl sulfate (SDS), β-nicotinamide adenine dinucleotide (NAD^+), coomassie brilliant blue, glutathione, and bovine serum albumin were purchased from Sigma–Aldrich (St. Louis, MO). Ethanol, acetaldehyde, benzaldehyde, ethylenediaminetetraacetic acid (EDTA), and Folin–Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany).

2.2. Expression and purification of human ADH and ALDH

The expression of recombinant enzymes in *Escherichia coli* and purification to apparent homogeneity for human ADH1A, ADH1B1, ADH1B2, ADH1B3, ADH1C1, ADH1C2, ADH2, ADH3, ADH4, and for human ALDH1A1, ALDH2, ALDH3A1 were carried out as described previously [6,15,29]. 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 SDS–polyacrylamide gel electrophoresis. Protein concentration was determined by the Lowry method [30] using bovine serum albumin as the standard.

2.3. 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 EDTA (only for ALDH assay), and varied concentrations of substrate and inhibitor. The cytosolic NAD^+ concentration in rat hepatocytes is reported to be ca. 0.5 mM [31]. Stock solutions of aspirin and salicylate were prepared in 0.1 M sodium phosphate, pH 7.5 and 25 °C. The aspirin stock was freshly prepared every 3 h during kinetic experiments to keep minimal hydrolysis (less than 3%) to salicylate. The concentration of hydrolytic product salicylate in aspirin stock solution following storage was determined fluorometrically as described previously [28]. 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 ADH and ALDH assay (unless otherwise indicated), or at 460 nm for emission of the fluorescence for the assay of ADH3 (excitation at 340 nm), ALDH1A1 and ALDH2 (excitation at 360 nm for the latter two to avoid interference of salicylate). Preparation of formaldehyde and calculation of the concentration of *S*-hydroxymethylglutathione from the equilibrium constants were performed as described previously [32]. 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 [33]. Initial velocity data were fitted with HYPER program to the Michaelis–Menten equation

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

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

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

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

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

where V_{max} is the maximal 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, respectively. The type of inhibition was determined by evaluating the standard errors of the kinetic constants and the residual variance for the equation that best fit the data [33]. In cases where the intercepts and slopes did not vary greatly with inhibitor concentration, Student's *t*-tests were applied to determine if they were significantly different. The kinetic experiments were performed in duplicate with five substrate concentrations usually ranging from 0.5 to 5 K_m and five (including one for control, $I = 0$) inhibitor concentrations ranging from 0.2 to 2 K_i when applicable. Values represent means ± standard error of the mean (SEM). Standard errors of the fits to the appropriate computer programs were less than 10% of the values for K_m and V_{max} and less than 16% of those for the inhibition constants, indicating good precision.