Substrate Specificity of Human Cutaneous Alcohol Dehydrogenase and Erythema Provoked by Lower Aliphatic Alcohols*

Jonathan K. Wilkin, M.D. and James H. Stewart, Ph.D.

Dermatology Section, McGuire Veterans Administration Medical Center, Richmond, and Departments of Pharmacology and Dermatology, Medical College of Virginia, Richmond, Virginia, U.S.A.

The substrate utilization rates of human cutaneous alcohol dehydrogenase were determined for 7 lower aliphatic primary alcohols: ethanol, propanol, butanol, pentanol, 2-methylpropanol, 3-methylbutanol, and 2,2-dimethylpropanol. 1-Pentanol gave the highest relative activity and 2,2-dimethylpropanol the lowest. The frequency of erythemogenesis was determined in vivo for these 7 lower aliphatic primary alcohols. The frequency of erythemogenesis correlated strongly and significantly with the rate of substrate utilization by alcohol dehydrogenase. These results are consistent with the view that the reaction to primary alcohols applied topically to human skin is provoked, in large part, by the corresponding aldehyde. *J Invest Dermatol 88:452–454, 1987*

There is increasing evidence for a genetic contribution to alcoholism [1–3]. Such genetic factors may operate through enzymatic mechanisms, influencing the metabolism of ethanol and its product, acetaldehyde. Higher blood levels of acetaldehyde occur after ethanol administration both in alcoholic vs nonalcoholic subjects and in subjects with alcoholic parents or siblings vs matched controls with no familial alcoholism [4,5]. Further, subjects with family histories of alcoholism flush more than matched controls, and the flushing response correlates positively and significantly with blood acetaldehyde level [6].

Moreover, there is a significant direct relationship between blood acetaldehyde levels after alcohol intake and facial flushing in Orientals [7]. Since alcohol dehydrogenase is the principle and rate-limiting enzyme of ethanol metabolism, genetic variation in expression of alcohol dehydrogenase isoenzymes in human liver could provide the basis for individual and racial differences in rates of alcohol metabolism [8]. Accordingly, it has been suggested that Oriental alcohol flushing is due to increased acetaldehyde formation in individuals with an "atypical" superactive alcohol dehydrogenase [9].

Not only is there an enhanced vascular sensitivity to ingested ethanol in Orientals, but there is also a predisposition to urticaria and erythema provoked by lower aliphatic primary alcohols and aldehydes [10,11]. Further, it is likely that the reaction to primary alcohols is actually provoked by the corresponding aldehyde [11]. Since cutaneous alcohol dehydrogenase may have a major role in the cutaneous vascular sensitivity to lower aliphatic alcohols, we compared the relative substrate specificity with frequency of erythemogenesis.

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Reprint requests to: Jonathan K. Wilkin, M.D., Chief, Dermatology Section (111L), McGuire VAMC, 1201 Broad Rock Road, Richmond, Virginia 23249.

MATERIALS AND METHODS

Human Skin Strips of skin were obtained in the operating room from the grossly normal portion of 7 below-knee amputation specimens. The strips were washed with normal saline, trimmed of subcutaneous fat, and stored at −20°C. The frozen strips were thinly sliced and then 2-g specimens were homogenized in 6 ml 0.01 M Na-K phosphate buffer, pH 7.4, with an Ultra Turrax homogenizer for 1 min at 0–4°C. Homogenates were centrifuged in a Sorvall RC-5 centrifuge, at 25,000 *g* for 20 min at 0–4°C. The supernatant was transferred to another tube, which was kept on ice until enzyme assay.

Enzyme Assay Alcohol dehydrogenase activity was assayed in 50 mM glycine buffer at a pH of 10.7 at 25°C. The reaction mixture contained 2.4 mM NAD and 1 nM lower aliphatic primary alcohol in each 1.0-ml assay specimen. The alcohol dehydrogenase activity was determined by the measurement of change in optical density at 340 nM [12]. Protein concentration of skin homogenates was determined by the method of Bradford [13]. Enzyme activity was expressed as the amount of NADH generated, viz, nM/mg protein-min ± SEM. The relative activity (ethanol = 1.0) was calculated to facilitate comparisons.

Human Subjects Twelve clinically normal subjects, comprising students, faculty, laboratory technicians, and their spouses, participated in this study approved by the local institutional review board.

Erythema Testing Acute patch testing to ethanol, propanol, butanol, pentanol, 2-methylpropanol, 3-methylbutanol, and 2,2-dimethylpropanol were tested at a concentration of 75% in aqueous solution. Volumes of 25 μl were pipetted onto individual coarse-porosity, quantitative, ashless grade filter paper squares placed on the volar forearms of the subjects after immersing the subjects' forearms in water at 33°C for 10 min followed by gently blotting dry. The patches were covered with Parafilm M for 5 min and

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then removed. The area was gently blotted. Those sites showing erythema during the subsequent 60 min are described as positive, and those sites without erythema are described as negative.

Statistical Analysis The frequency of erythemogenesis vs the relative activity of the lower aliphatic primary alcohol is analyzed by linear regression employing the method of least squares.

RESULTS

The alcohol dehydrogenase activity employing different lower aliphatic primary alcohols as substrates of the crude homogenates varies from virtually no activity to 3 times that of ethanol (Table I). Similarly, 2 lower aliphatic primary alcohols produced erythema in only 2 subjects, whereas 3 lower aliphatic primary alcohols produced erythema in all 12 subjects (Table I). There was a significant correlation between the relative alcohol dehydrogenase activity and frequency of erythemogenesis among these 7 lower aliphatic primary alcohols ($r = 0.882$) (Fig 1).

DISCUSSION

Since the rate of substrate utilization correlates with erythemogenesis, these results are consistent with the view that the reaction to primary alcohols is actually provoked, in large part, by the corresponding aldehyde [11]. Importantly, alcohol dehydrogenase is not specific for ethanol, since the oxidation of propanol, 2-pentanol, and butanol is also catalyzed. However, only primary alcohols are oxidized to corresponding aldehydes by this enzyme; secondary and tertiary alcohols are not.

Furthermore, the evidence supporting the role of aldehydes in this ethnic cutaneous vascular sensitivity to lower aliphatic alcohols includes several observations [11]. First, only primary alcohols, which can be oxidized to the corresponding aldehyde, and not secondary or tertiary alcohols, will elicit cutaneous erythema. Second, the reaction to primary alcohols can be totally blocked by pretreatment with a potent inhibitor of alcohol dehydrogenase. Third, the reaction to acetaldehyde occurs earlier than the reaction to ethanol, which is compatible with a time lag requisite for conversion of ethanol to acetaldehyde. Fourth, aldehydes routinely provoke cutaneous erythema. Finally, in this study we have shown that the rate of conversion to the cognate aldehyde correlates significantly with the erythemogenicity of the lower aliphatic primary alcohols.

In normal Orientals ethanol-provoked flushing occurs with a conspicuous rise in blood acetaldehyde levels. In fact, a significant elevation of blood acetaldehyde does not occur in Orientals who do not flush after consuming ethanol. Insofar as the direct factor is acetaldehyde, the cutaneous model of the Oriental sensitivity to alcohol is quite congruous with the systemic reaction.

Although alcohol sensitivity in Orientals due to increased acetaldehyde formation was originally attributed to an atypically superactive alcohol dehydrogenase [9], subsequent studies focused on an atypically sluggish aldehyde dehydrogenase isoenzyme [14–16]. Erythrocyte aldehyde dehydrogenase activity correlates

Table I. Comparison of Alcohol Dehydrogenase Activity and Erythemogenesis of Lower Aliphatic Primary Alcohols

Alcohol	Enzyme Activity (nM/mg protein-min)	Relative Activity (Ethanol = 1.0)	Frequency of Erythemogenesis (n = 12)
Ethanol	98.1 ± 37.0[a]	1.0 ± 0.4[a]	41.7%
1-Propanol	166.1 ± 62.7	1.7 ± 0.6	75.0%
1-Butanol	234.7 ± 69.9	2.4 ± 0.7	100.0%
1-Pentanol	300.9 ± 85.7	3.1 ± 0.9	100.0%
2-Methylpropanol	103.7 ± 40.7	1.1 ± 0.4	16.7%
3-Methylbutanol	183.3 ± 60.0	1.9 ± 0.6	100.0%
2,2-Dimethylpropanol	0.0 ± 9.6	0.0 ± 0.1	16.7%

[a]SEM.

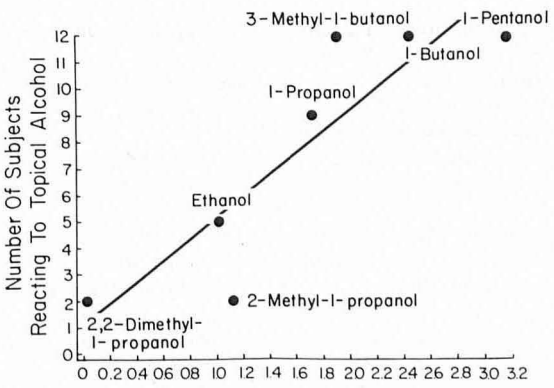

Figure 1. Number of subjects (n = 12) reacting to topical alcohols with different relative activities as substrates for human cutaneous alcohol dehydrogenase (ethanol = 1.0).

significantly with blood acetaldehyde levels and the cardiovascular response to alcohol, and has been proposed as a marker for alcohol sensitivity in Orientals [17].

Moreover, sluggish aldehyde dehydrogenase activity due to an atypical isoenzyme has been regarded as the cause of flushing in Japanese after ingesting ethanol [18,19]. The additional factor of superactive alcohol dehydrogenase could elevate the acetaldehyde level, thus compensating for the decrease in aldehyde dehydrogenase activity, and maintain the rate of ethanol elimination unchanged in alcohol-sensitive individuals [20]. In this setting, the acetaldehyde level should be greater still. Accordingly, it has been suggested that the most probable cause of alcohol flushing in Orientals is a combination of atypically superactive alcohol dehydrogenase and atypically sluggish aldehyde dehydrogenase [21]. That the most important factor is the atypically superactive alcohol dehydrogenase is supported by the observation that the frequency of alcohol sensitivity among different ethnic groups matches the frequency of atypical alcohol dehydrogenase, but not the frequency of atypical aldehyde dehydrogenase, in these populations [22].

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