

Impaired acetaldehyde oxidation in alcoholics*

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SUMMARY High blood acetaldehyde levels in alcoholics after ethanol ingestion are due to reduced acetaldehyde oxidation rather than to an increased rate of its formation from ethanol. This is associated with low hepatic acetaldehyde dehydrogenase activity in alcoholic subjects and may represent a specific abnormality in them.

Acetaldehyde, which is formed during ethanol metabolism in the liver, is extremely toxic. It has been suggested as an important agent in the pathogenesis of alcoholic liver disease,¹⁻³ and as a basis for alcohol addiction.^{4,5} After drinking ethanol most acetaldehyde is not only formed in the liver, but is also immediately oxidised there, so that little enters the blood in normal subjects.^{6,7} In alcoholics given infusions of ethanol, however, higher blood levels of acetaldehyde have been reported.^{7,8} Both a 'vicious cycle' theory of alcoholic liver injury mediated by acetaldehyde⁹ and a primary abnormality of acetaldehyde metabolism predisposing to alcoholism¹⁰ have been suggested to explain this difference, but the reason for it remains unclear, and the original studies have been criticised because the methods used to measure acetaldehyde were inadequate.¹¹

More acetaldehyde may leave the liver in the blood if the rate of its formation from ethanol increases, or if the liver's capacity to oxidise acetaldehyde falls. In alcoholics both mechanisms are possible, as the rate of ethanol metabolism may be increased^{12,13} and the activity of hepatic acetaldehyde dehydrogenase (acetaldehyde dehydrogenase) is reduced.¹⁴ In the majority of biochemical reactions, however, enzyme activity is not the rate-limiting factor, so that a reduction in hepatic acetaldehyde dehydrogenase activity may not necessarily limit acetaldehyde oxidation in the liver, just as alcohol dehydrogenase activity is not rate-limiting for the oxidation of ethanol.^{15,16}

In this study both these possible mechanisms were

investigated. Alcoholic and control subjects were given ethanol orally, and the concentrations of ethanol and acetaldehyde in the blood were measured at intervals afterwards. In addition, the specific activity of acetaldehyde dehydrogenase was assayed in liver biopsies from some of the alcoholic patients.

As the reduced specific activity of hepatic acetaldehyde dehydrogenase observed in the alcoholic patients might be a non-specific consequence of liver damage, acetaldehyde dehydrogenase activity was also assayed in liver biopsies from patients with other forms of liver disease, and in some other control subjects.

NAD is an essential cofactor for acetaldehyde oxidation in the liver, so that hepatic NADH/NAD ratio is another possible influence at this reaction. The lactate/pyruvate ratio in the blood reflects the dramatic change in hepatic NADH/NAD ratio which occurs during ethanol metabolism.¹⁷ It was therefore determined in some of the patients during the ethanol-loading study.

Methods

Seventeen male alcoholic subjects and nine healthy male control subjects were studied. Their clinical and biochemical details are shown in the Table. All the alcoholic subjects had regularly consumed more than 80g ethanol daily for at least two years, and had continued to do so until 48 hours before the study.

After an overnight fast each subject drank 1g ethanol/kg body weight as a 10% solution (w/v) in orange juice over 10 minutes. Venous blood samples for the estimation of ethanol and acetaldehyde were taken through an indwelling siliconised cannula before and for six hours after the ethanol was drunk. Samples were taken every 15 minutes for two hours, and then every 30 minutes. Blood ethanol and

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Table Clinical and biochemical data of subjects in ethanol-loading study

Histology	Number	Age (yr)	Mean + SD			
			AST (IU/l)	Albumin (g/l)	GGT (IU/l)	MCV (fl)
Controls	9	33 (5)	15 (5)	46 (4)	38 (15)	85 (4)
Fatty liver	9	49 (9)	33 (12)	46 (4)	155 (5)	99 (5)
Alcoholic hepatitis	5	46 (8)	57 (24)	46 (4)	198 (80)	106 (14)
Alcoholic cirrhosis	3	58 (10)	70 (50)	37 (2)	485 (146)	99 (8)
Normal range			5-15	35-50	10-48	80-95

acetaldehyde were assayed simultaneously by the head-space gas chromatography method of Brien and Loomis.¹⁸ This method gives similar results to the more complex semicarbazide method of Stowell.¹⁹ It prevents the non-enzymatic formation of acetaldehyde from ethanol in blood, and gives an 84% recovery of 22 $\mu\text{mol/l}$ acetaldehyde (final concentration) added to blood.

The rates of ethanol oxidation,²⁰ and the mean blood acetaldehyde concentrations were calculated in control and alcoholic subjects, and the differences between the two groups were analysed using the Wilcoxon rank test.

Blood lactate and pyruvate concentrations were assayed by standard methods^{21, 22} in 10 of the alcoholic patients before, and 60 and 90 minutes after the ethanol was drunk.

HEPATIC ACETALDEHYDE DEHYDROGENASE ACTIVITY

The specific activity of acetaldehyde dehydrogenase was measured in liver biopsies from 10 of the patients who took part in the ethanol-loading study, and in 20 other patients with alcoholic liver disease. It was also assayed in eight patients with other forms of liver disease, and in 10 subjects undergoing diagnostic liver biopsy to exclude liver disease, but who had normal serum liver function tests at the time, and whose biopsies were subsequently shown to be histologically normal.

Acetaldehyde dehydrogenase activity in the liver biopsies was assayed by a gas chromatographic method.²³ It involves repeated measurement of acetaldehyde in the head-space gas above an incubation mixture contained within a sealed vial. This method gives values for the specific activity of hepatic acetaldehyde dehydrogenase similar to those obtained by a standard spectrophotometric assay,¹⁴ if the same concentration of acetaldehyde is used.²³ In this study a low concentration of acetaldehyde (23 $\mu\text{mol/l}$) was used to increase the precision of the assay. Consequently the specific activities of hepatic acetaldehyde dehydrogenase are lower than those previously reported.¹⁴ Protein has been shown previously to be a satisfactory reference

for the specific activity of hepatic enzymes in alcoholic liver disease.²⁴ It was measured by the Lowry method.²⁵

Results

Figure 1 shows the blood ethanol and acetaldehyde concentrations measured at intervals after the ethanol was drunk in the control and alcoholic groups. Although the peak values of blood ethanol were slightly higher in the alcoholic subjects, the mean rate of ethanol oxidation (132 ± 28 (SD) mg/h/kg) did not differ significantly from that in control subjects (130 ± 30 mg/h/kg). In contrast, blood acetaldehyde concentrations were significantly higher in the alcoholic group than in the control group at all time points except 120 minutes. The mean blood acetaldehyde concentrations over the six hour period after ethanol loading were 17.8 ± 6 (SD) $\mu\text{mol/l}$ in the alcoholic subjects and

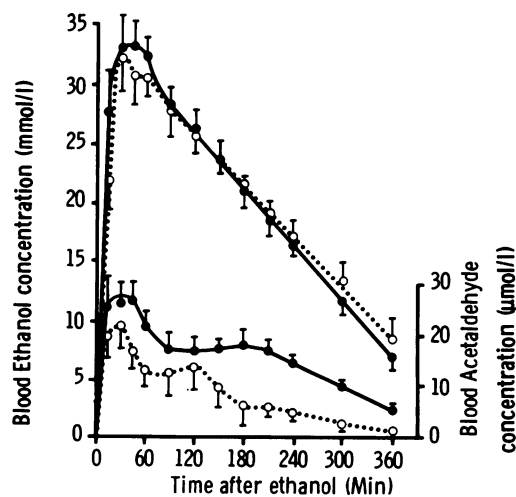


Fig. 1 Blood ethanol and acetaldehyde concentrations (mean \pm SEM) in alcoholic ($n=17$) and control nine subjects. (1 mmol/l ethanol = 4.6mg%).

10.0±4 μmol/l in the controls (p<0.01). The mean blood acetaldehyde concentration after drinking ethanol did not vary significantly with age in either control or alcoholic subjects, and in the alcoholic group it did not correlate with either abnormal serum liver function tests or the histological severity of the liver damage.

In the alcoholic patients, however, the specific activity of hepatic acetaldehyde dehydrogenase was significantly lower (p<0.01) than in both normal subjects of a similar age (mean 46.3±11 (SD) years), and patients with other forms of liver disease (mean age 52.5±6 years) (Fig. 2). There was also a significant inverse relationship (p<0.05) between the specific activity of acetaldehyde dehydrogenase in the liver biopsies from the alcoholic patients and the mean concentration of acetaldehyde in their blood after drinking ethanol (Fig. 3).

There was a similar inverse relationship between the mean blood lactate/pyruvate ratio 60 and 90 minutes after ethanol and the mean blood acetaldehyde concentrations (Fig. 4).

Discussion

This study confirms that recently drinking alcoholics have higher concentrations of acetaldehyde in

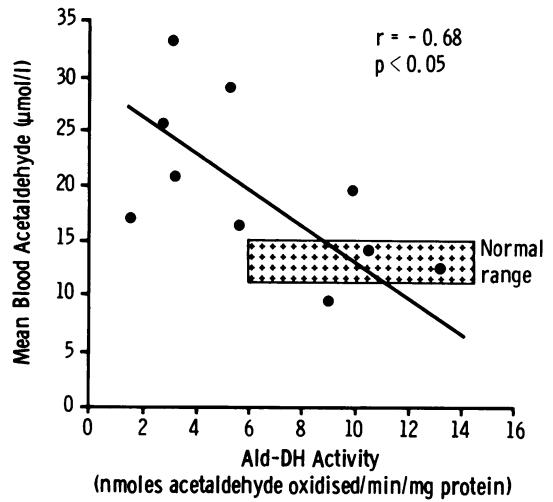


Fig. 3 Relationship between mean blood acetaldehyde concentration and hepatic acetaldehyde dehydrogenase activity in 10 alcoholic subjects.

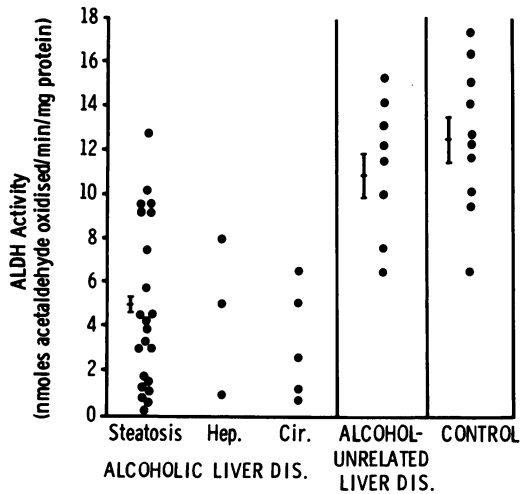


Fig. 2 Hepatic acetaldehyde dehydrogenase activities in biopsies from alcoholic, control, and alcohol-unrelated liver disease subjects with mean ± SEM for each group. (The alcohol-unrelated liver disease group comprised chronic active hepatitis (two subjects), cryptogenic cirrhosis (two), intrahepatic cholestasis (two), drug reactions (one), and primary biliary cirrhosis (one)).

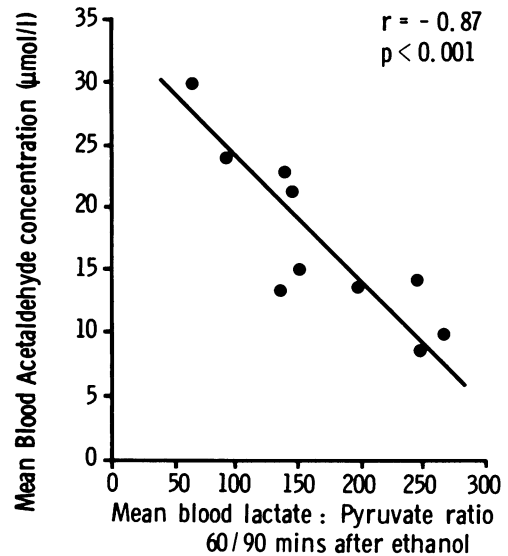


Fig. 4 Relationship between mean blood acetaldehyde concentration and mean blood lactate/pyruvate ratio after ethanol in 10 alcoholic subjects.

venous blood after drinking ethanol than normal subjects. This difference does not result from an increased rate of acetaldehyde production from ethanol in the alcoholics, as the rates of ethanol oxidation were similar in the alcoholic and control groups (Fig. 1). The higher blood levels in the alcoholics are probably due to reduced oxidation of acetaldehyde in the liver, where the majority of acetaldehyde formed from ethanol is normally metabolised.

The hepatic activity of acetaldehyde dehydrogenase has previously been shown to be reduced in alcoholics,¹⁴ and numerous animal experiments have suggested that its activity in the liver is rate-limiting for hepatic oxidation of acetaldehyde.²⁶⁻²⁹ Hepatic acetaldehyde dehydrogenase activity is probably rate-limiting for the oxidation of acetaldehyde in human liver too, as there was a significant inverse relationship between the mean venous blood acetaldehyde concentration after ethanol and the acetaldehyde dehydrogenase activity assayed in liver biopsies from our alcoholic patients (Fig. 3). In contrast, the inverse relationship between the mean venous blood acetaldehyde concentration and the blood lactate/pyruvate ratio after ethanol administration (Fig. 4) indicates that the hepatic redox state does not regulate acetaldehyde metabolism in the liver, as a positive correlation would be expected if it did. Similar results have been obtained in animal studies.^{27 30}

Whether the reduction in hepatic acetaldehyde dehydrogenase activity in alcoholics represents a primary abnormality, or is secondary to liver damage remains unknown. The lack of an association between reduced hepatic acetaldehyde dehydrogenase activity and either abnormalities of serum liver function tests, or histological severity of the liver lesion in the alcoholics (Fig. 2), and the finding of almost normal levels of activity in other forms of liver disease, however, lead us to suggest that reduced hepatic acetaldehyde dehydrogenase activity may represent a primary abnormality at least in some alcoholics. Such a primary reduction in hepatic acetaldehyde dehydrogenase activity may go some way in explaining the inheritance of impaired acetaldehyde metabolism previously reported in the healthy non-alcoholic sons of alcoholic fathers.¹⁰

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