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Plasma Alpha Amino-*n*-Butyric Acid to Leucine Ratio: An Empirical Biochemical Marker of Alcoholism

Abstract. *The plasma ratio of α-amino-*n*-butyric acid to leucine was elevated in ambulatory and hospitalized alcoholics as well as in baboons fed alcohol along with an adequate diet. There was a statistically significant positive correlation between this ratio and the degree of alcoholism assessed by three separate medical and psychological criteria in patients maintained on methadone.*

Alcoholism has been defined by many criteria (physiological, clinical, behavioral, psychological, attitudinal), but a universally accepted or practical definition is not available. Recently, we observed in alcoholics characteristic alterations in the plasma amino acids. The branched-chain amino acids (BCAA) were significantly depressed, whereas the concentration of α-amino-*n*-butyric acid was increased relative to the BCAA (*1*). The present study was undertaken to establish whether the ratio of plasma α-amino-*n*-buytric acid to leucine (A/L) could serve as a biochemical marker of long-term heavy drinking. Such a marker would enable the objective evaluation of different treatment modalities for alcoholism and would provide a means for the early detection and treatment of alcoholism. The study was conducted with 42 hospitalized alcoholics, 20 control subjects, 19 patients with nonalcoholic liver disease, and 25 participants in a methadone maintenance program. Alcohol was also fed to 13 baboons (as 50 percent of the total calories), and these were compared with 13 pair-fed control animals.

The 42 hospitalized alcoholics fulfilled the major criteria of alcoholism as defined by the National Council on Alcoholism (NCA) (*2*), and the majority had been drinking heavily until the time of admission. Blood was obtained after an overnight fast 1 to 60 days following admission. The blood was deproteinized with sulfosalicylic acid and analyzed for amino acids with a Beckman 119 amino acid analyzer according to the two-column method for physiological solutions (*3*). Twenty nonalcoholics (seven laboratory workers, seven patients, and six methadone maintenance patients) served as a common control group. Plasma ratios of A/L were determined for each subject, and the means for alcoholics and nonalcoholics were calculated. The hospitalized alcoholics had a greater than twofold increase in the ratio of A/L compared to the nonalcoholic controls (Fig. 1). A similar increase was observed in nine of the baboons fed alcohol (along with an adequate diet) (*4*) for 1 to 4 years. Furthermore, an increased ratio was present both in 14 hospitalized alcoholics and three baboons that had only minimal biochemical or morphological hepatic abnormalities due to alcohol, as well as in the remaining 28 patients and six baboons with more severe liver injury. However, no elevation of the A/L ratio was noted in 19 patients with nonalcoholic liver injury.

We observed an abnormally elevated ratio both in well-nourished alcoholics and in poorly nourished ones. Indeed,

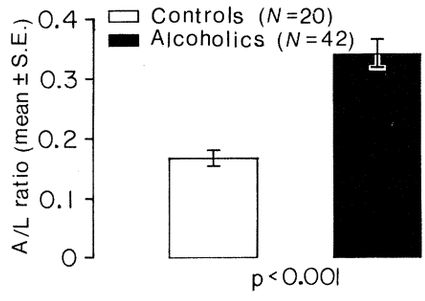

Fig. 1. Increased plasma A/L ratios in a representative population of hospitalized alcoholics. Alcoholism was defined by the fulfillment of major NCA criteria (*2*).

the ratio of A/L (mean ± standard error) among alcoholics with dietary protein deficiency for greater than 2 weeks (N = 21; A/L = 0.347 ± 0.039) was similar to that in alcoholics with deficiencies of shorter duration (N = 21; A/L = 0.350 ± 0.026), both ratios being high compared to controls (N = 20; A/L = 0.166 ± 0.011). Baboons fed alcohol along with a protein deficient diet (6 percent of calories as protein) developed an abnormal ratio (N = 4; A/L = 0.250 ± 0.054) when ethanol was given but not when it was omitted (N = 4; A/L = 0.090 ± 0.017). Furthermore, it was reported previously (*5*) that in extreme dietary protein deficiency (kwashiorkor), the calculated mean A/L ratio was within 2 standard deviations (S.D.) of the mean of our nonalcoholic controls.

The mechanism whereby long-term alcohol consumption results in plasma amino acid abnormalities is unknown. Extreme abnormalities of carbohydrate metabolism have been described in which the calculated ratio of A/L is increased: for example, in patients with massive obesity undergoing starvation (*6*) and in subjects consuming an experimental diet with very low carbohydrate and high fat content (*7*). The relationship of these findings to the abnormal ratios we observed in alcoholics is unknown since none of the subjects we studied displayed these severe disturbances. However, the possibility that other metabolic abnormalities, especially those of carbohydrate metabolism, increase the plasma A/L ratio and result in false positive tests warrants further study. The effects of a single large dose (1 g/kg) of ethanol on this amino acid ratio were investigated in four subjects. The mean ratio of A/L did not increase; actually it decreased progressively from 0.173 to 0.121 over an 8-hour period following ingestion. Only two of the 42 hospitalized alcoholics had measurable alcohol in the blood at the time of sampling and thus the presence of alcohol in the blood is not required for the positivity of this test. The effect of the time elapsed since long-term alcohol consumption on this ratio was assessed by comparing patients sampled within 1 week of cessation of drinking with patients sampled after this period. An elevated ratio was defined as being greater than 2 S.D. above the means for controls. In subjects sampled within 1 week of cessation of drinking 16 out of 18 had elevated ratios while in those sampled after this period 13 out of 24 had elevated ratios. Furthermore, all baboons receiving ethanol on a long-

term basis had elevated ratios when sampled after an overnight fast. This test therefore reflects prolonged ethanol intake rather than ethanol ingestion over a short period, and its positivity persists for a week or more beyond the long-term drinking period but decreases with prolongation of abstinence.

The A/L ratio was next investigated as a marker to detect and assess alcoholism in a population of 25 patients maintained on methadone. This population was under close counselor supervision, and included nonalcoholics and a minimum of peer and employee pressure to conceal excessive drinking. Except for alcohol and previous narcotic abuse, no individual had significant medical problems. Each subject was assessed for alcoholism by his counselor with respect to NCA criteria of alcoholism after at least 6 months of observation. Of these 25 patients, 19 took the Swenson and Morse self-administered alcoholism screening test (SAAST) (*8*). All 25 underwent a confidential interview in which a physician assessed their average daily ethanol intake; consistent results were obtained and utilized in 18 of the subjects. A blood specimen, obtained in 20 subjects following breakfast and in 5 subjects following an overnight fast, was analyzed for amino acids as described before. In addition, each subject had undergone recent serum chemistry measurements including serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase, and bilirubin, as well as a blood ethanol determination performed on the sample obtained for amino acid analysis. All subjects who consumed ethanol daily had been drinking until the time of sampling.

A positive correlation between the plasma A/L ratio and the average daily intake of ethanol, SAAST test scores, and NCA criteria (on a scale of 0 to 4: no criteria satisfied = 0, minor criteria satisfied = 1 to 2, major criteria satisfied = 3 to 4) was determined on a Hewlett-Packard calculator programmed for linear regressions (*9*). A patient was defined as alcoholic if the major NCA criteria were met, the SAAST test scores were 10 or more, and if more than 2 g of ethanol per kilogram were injested per day. Failure to fulfill NCA major and minor criteria, SAAST test scores of less than 7, and ethanol intakes of less than 0.2 g per kilogram per day were taken to indicate the absence of alcoholism. A ratio was defined as being elevated if it were more than 2 S.D. above the mean of 20 control patients assessed as nonalcoholic by both NCA and intake criteria.

Table 1. Incidence of elevated A/L ratios among alcoholics (as defined by other criteria) in a group of 25 participants in a methadone maintenance program. A positive determination was defined as a ratio greater than 2 S.D. above the mean of a control group consisting of 20 subjects assessed as nonalcoholic by NCA and intake criteria.

Criteria of alcoholism	Number of positive determinations	Number of subjects
NCA (major criteria)	6	9
SAAST (test score $\geq$ 10)	7	11
Intake ($> 2\ g\ kg^{-1}\ day^{-1}$)	5	6
NCA + SAAST + intake	4	4

A statistically significant positive correlation was observed between the plasma A/L ratio and the degree of alcoholism assessed by each of three criteria: NCA, $r = 0.522$, $P < .01$; SAAST, $r = 0.532$, $P < .05$; and intake, $r = 0.522$, $P < .05$. The ability to detect alcoholics was investigated by measuring the frequency of elevated ratios among alcoholics assessed by each of the three criteria. As shown in Table 1, the plasma A/L ratio was elevated in the majority of alcoholics identified by each of the three individual criteria and in all four subjects in whom all criteria were fulfilled. By contrast, ethanol was present in the blood in only a minority of alcoholics as assessed by each of the three criteria and in only two out of four subjects in whom all criteria were fulfilled. Similarly, the presence of alcohol in the blood has been reported in only a minority of heavy drinkers or outpatients with liver injury related to alcohol ingestion (*10*). Because the presence of alcohol in the blood does not distinguish short-term from long-term alcohol consumption, the plasma A/L ratio is a more reliable marker of alcoholism than is blood alcohol. Similarly, the plasma A/L ratio is more valuable than blood enzyme changes. Indeed, although 11 out of 25 subjects had asymptomatic minimal elevations of SGOT, SGPT, and alkaline phosphatase, there was no significant correlation between these abnormalities and the degree of alcoholism as assessed by each of the three criteria. Other studies have reported elevations of serum enzymes such as glutamic dehydrogenase and γ-glutamyl transpeptidase in only a fraction of "heavy drinkers" (*11*) and these enzymes may also be increased in the presence of liver injury that is not caused by alcoholism. Indeed, in our study, all 19 nonalcoholic subjects with liver injury had abnormally elevated serum chemistries while none had an elevated A/L ratio. The plasma A/L ratio was elevated in the majority of the alcoholics maintained on methadone who were predominantly nonfasting as well as in hospitalized alcoholics who were fasting. Thus, a positive test does not depend upon the presence or absence of fasting.

There were no false positive elevations of the plasma A/L ratio among six patients maintained on methadone and assessed as nonalcoholic by NCA criteria, nor among six such patients assessed as nonalcoholic by intake criteria. Only one subject had an elevated ratio among six subjects defined as nonalcoholics by SAAST criteria. It should be noted, however, that this individual fulfilled NCA minor criteria of alcoholism.

We conclude that the A/L ratio increases in relation to long-term alcohol consumption. Furthermore, this ratio correlates positively with the degree of alcoholism as assessed by separate criteria in a population of active alcoholics and it can identify the majority of alcoholics within that population. The plasma A/L ratio may therefore become an objective empirical marker for the detection and assessment of alcoholism.

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