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Frequency of Hyperprolinemia in Alcoholic Liver Cirrhosis: Relationship to Blood Lactate

SPENCER SHAW, THERESA M. WORNER, AND CHARLES S. LIEBER

Section of Liver Disease and Nutrition, Alcohol Research and Treatment Center and Kingsbridge Research Foundation, Veterans Administration Medical Center, Bronx, New York 10468, and Mount Sinai School of Medicine (CUNY), New York, New York 10029

In alcohol-induced liver injury, hyperprolinemia has been proposed as a marker of fibrogenesis probably secondary to hyperlactacidemia. However, some studies of plasma proline in alcoholics with cirrhosis have reported normal rather than increased levels. In order to evaluate the frequency of hyperprolinemia in alcoholic liver disease and its relationship to blood lactate, we measured plasma proline levels in 145 subjects including 91 alcoholics with a spectrum of liver disease as well as 22 nonalcoholics with liver injury unrelated to alcohol. We also studied baboons fed alcohol as 50% of total calories for 1 to 4 years. Among alcoholics only 21/91 had elevated proline values. Elevations were most frequent among patients with severe hepatic decompensation (3/8), in patients with alcoholic hepatitis on liver biopsy (5/17), and in those with acute alcohol-related withdrawal, with alcohol still present in the blood (4/9). Patients with liver disease unrelated to alcohol but severe in nature also has elevations in proline (3/3). Only 4/28 patients with cirrhosis due to alcohol had elevated values, and none of the baboons fed alcohol had hyperprolinemia whether withdrawn from alcohol or not. Hyperlactacidemia was associated with hyperprolinemia, but so were depressed serum albumin values and prolongations of the prothrombin time, suggesting a general association with severe liver disease. These results reveal that hyperprolinemia occurs infrequently in patients with alcohol-induced cirrhosis and therefore does not appear to be a sensitive marker of hepatic fibrosis in these patients.

Hyperprolinemia has been reported as a specific and significant feature of alcoholic liver cirrhosis (1, 2). It has been postulated that the increased proline in the blood is due to altered hepatic metabolism of proline and may be a marker of fibrogenesis in alcoholic liver disease. An increase in the hepatic proline pool is reported in cirrhosis (3), and the latter has been found in *in vitro* studies to be important for the regulation of collagen synthesis (4). While the precise mechanism of the hyperprolinemia observed in such patients is unknown, it was found in some studies to be associated with (and postulated to be a consequence of) hyperlactacidemia.

Several conditions unrelated to alcohol-induced cirrhosis may also lead to hyperprolinemia including hepatic necrosis of viral etiology (5), sepsis (6), and lactic acidosis (7). Furthermore, a number of studies in patients with alcohol-induced cirrhosis (8–11) or with earlier stages of alcoholic liver injury (12, 13) have reported normal plasma proline levels. Thus, in order to clarify the frequency of hyperprolinemia in alcohol-induced cirrhosis, its specificity in this disease, and possible mechanisms of hyperprolinemia when it occurs, we studied 145 subjects including 91 alcoholics and 54 nonalcoholics as well as subhuman primates (baboons) fed alcohol as 50% of total calories for 1 to 4 years.

MATERIALS AND METHODS

SUBJECTS

Controls. The control group was composed of 32 subjects; 8 patients admitted for reasons unrelated to alcohol or liver disease, and 24 laboratory workers and medical staff. None had a history of liver disease or clinical or laboratory evidence (SMA-18) of liver disease. Each consumed less than the equivalent of 10 gm of absolute ethanol per day, and none had alcohol measurable in the blood in a fasting a.m. blood sample. No subject had a previous history of alcohol abuse or alcohol related problems.

Alcoholics. Ninety-one alcoholics admitted to the Medical Service at the Bronx Veterans Administration Medical Center for alcohol-related withdrawal or medical complications of alcoholism were studied. Sixty-six of the 91 underwent liver biopsy as part of their clinical

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Address reprint requests to: Spencer Shaw, M.D., Alcohol Research and Treatment Center, Veterans Administration Medical Center, 130 W. Kingsbridge Road, Bronx, New York 10468.

management. The 25 that were not biopsied were admitted for alcohol-related withdrawal symptoms. Among the biopsied alcoholics 36 had cirrhosis, 17 had alcoholic hepatitis, 12 had steatosis (including perivenular fibrosis), and 1 had nonspecific changes in the liver. All biopsied patients except eight with severely decompensated cirrhosis were studied within 1 week of the time of biopsy. Patients in withdrawal were not biopsied and were studied within 72 hr of admission. Nine of 25 had ethanol in the blood at the time of the study.

Among the patients with cirrhosis, 8/36 were judged to have severe liver disease evidenced by persistent elevation of the prothrombin time (above 6 sec) despite vitamin K administration, depressed serum albumin (less than 3.5 gm per dl), and some evidence of hepatic encephalopathy.

Nonalcoholics with Liver Disease. Twenty-two patients with liver injury unrelated to alcohol were studied. None had a history of alcoholism or alcohol related medical problems. None had consumed alcohol within 2 weeks of the study. Twenty had liver biopsies performed as part of their clinical evaluation. Eight had acute viral hepatitis, 3 had chronic persistent hepatitis, 8 had chronic active hepatitis (including 3 with transition to cirrhosis), and 1 had metastatic adenocarcinoma to the liver. Two patients had jaundice, cholangitis, and sepsis and could not be biopsied because of elevated prothrombin times (more than 6 sec above control). These two patients and the one with metastatic liver disease all had hepatic encephalopathy, elevated prothrombin times, and depressed serum albumin levels (<3.5 gm per dl) at the time of the study and were categorized as having severe liver disease.

Baboons. Twenty-eight baboons were pair-fed diets containing either ethanol as 50% of total calories or the identical diet with ethanol replaced isocalorically by carbohydrate as described previously (14). Baboons were studied after 1 to 4 years of feeding. Eight of 14 alcohol-fed baboons had fibrosis or cirrhosis on biopsy at the time of study.

Sampling. All blood samples were obtained by simple venipuncture after an overnight fast. In the case of patients the blood obtained was that found to be in excess after routine drawing for clinical tests. Approximately 1 to 1.5 ml were used for each patient. In nine pairs of baboons, blood was obtained by venipuncture after an overnight withdrawal from alcohol and by simple venipuncture without anesthesia. An additional five pairs of baboons were studied after hepatic vein and femoral artery catheterization. Plasma proline in the hepatic vein and femoral artery was measured in the fasting state as well as 1 to 2 hr following intragastric intubation with 10 ml per kg or approximately one fifth of the respective daily diets.

Amino Acids

Blood for amino acids was placed in heparinized tubes and deproteinized with sulfosalicylic acid as described previously (15). Proline was measured on a Beckman 121 MB amino acid analyzer using the single column method for physiological solutions (16) with norleucine as an internal standard.

Blood Lactate Studies. In order to study the possible relationship between hyperprolinemia and hyperlactacidemia, blood lactates were measured on the venous blood samples of 65 of the patients as well as in the fasting samples in the baboons. Whole blood was deproteinized by a 1:3 dilution with 8% perchloric acid (w/v) and analyzed for lactate by an enzymatic method as detailed in Sigma Technical Bulletin No. 726 (17).

Blood Alcohol. Blood alcohol was measured by gas-liquid chromatography as described previously (18).

Other Laboratory Tests. Serum albumin, prothrombin time, SMA-18 (by Technicon Autoanalyzer), and liver histology in patients were performed in the Clinical Chemistry and Pathology sections of the hospital as part of the routine clinical management of the patients. Liver biopsies were independently reviewed to confirm the histological diagnosis. The liver histology of the baboons was determined on surgical biopsies obtained within 3 months of the time of study. Processing of samples was as described previously (19).

Statistics

Mean ± S.E. were determined for each of the values, and differences between groups were determined by Student's group t test. Correlation coefficients were determined by the least-squares method.

RESULTS

The frequency of elevated plasma proline values among alcoholics and nonalcoholics with liver disease is shown in Table 1. An elevated value was defined as one that exceeded the upper limit of normal of our 32 controls (280 μM), a value consistent with previously published normal values (2, 6, 8, 11, 12). Among all alcoholics only a minority (21/91) had elevations. Furthermore, among alcoholics with cirrhosis (without severe hepatic decompensation), less than 15% (4/28) had elevated proline values. Moreover, the mean value for this group was not significantly different from that of controls (Table 2).

A higher frequency of hyperprolinemia was observed in alcoholics with acute alcohol-related withdrawal (8/

Table 1. Frequency of Elevated Plasma Proline and Blood Lactate Values among Alcoholics and Nonalcoholics with Liver Disease[a]

	Plasma Proline >280 μM	Blood lactate >1.3 mM
Alcoholics		
All	21/91	15/48
Acute withdrawal		
Blood alcohol		
+	4/9	7/9
−	4/16	1/9
Steatosis—minimal liver changes	1/13	2/6
Hepatitis—fibrosis	5/17	3/9
Cirrhosis (moderate)	4/28	5/11
Cirrhosis (severe)	3/8	4/4
Nonalcoholics		
Moderate	0/19	1/7
Severe	3/3	3/3

[a] Only a minority of patients with alcoholic liver disease or nonalcoholic liver disease had elevations of either proline or lactate.

TABLE 2. PLASMA PROLINE AND BLOOD LACTATE LEVELS IN ALCOHOLICS AND NONALCOHOLICS WITH LIVER DISEASE

	Controls	Nonalcoholic liver disease		Alcohol withdrawal		Alcoholic liver disease			
				Blood alcohol		Fat (n = 13)	Hepatitis fibrosis (n = 17)	Cirrhosis	
		Moderate (n = 19)	Severe (n = 3)	(+) (n = 9)	(−) (n = 16)			Moderate (n = 28)	Severe (n = 8)
Proline (μM)	190 ± 8.4[a] (n = 32)	163.7 ± 13.2 NS[b,c]	903.6 ± 78.4 <0.001	421 ± 115 <0.05	231 ± 17 <0.05	180 ± 20 NS	266 ± 28 <0.02	225 ± 18 NS	334 ± 47 <0.001
Lactate (mM)	0.79 ± 0.23 (n = 7)	1.07 ± 0.33 NS	10.2 ± 4.0 <0.05	2.09 ± 0.25 <0.01	0.91 ± 0.16 NS	1.22 ± 0.19 NS	0.94 ± 0.18 NS	1.29 ± 0.12 NS	2.94 ± 0.86 <0.05
Albumin (gm/dl)	4.3 ± 0.11 (n = 32)	4.55 ± 0.17 NS	2.56 ± 0.54 <0.02	3.72 ± 0.29 NS	4.34 ± 0.09 NS	4.2 ± 0.19 NS	3.6 ± 0.27 <0.05	3.53 ± 0.15 <0.001	2.25 ± 0.09 <0.001
Prothrombin time elevations[d]	0/32 (n = 32)	0/19	3/3	2/8	0/16	0/13	2/17	3/29	8/8

[a] Mean ± S.E.
[b] Significance is expressed as the p value compared with controls.
[c] NS, not significant.
[d] More than 6 sec above control values.

25) and alcoholic hepatitis (5/17), but a low frequency was observed in alcoholics with merely steatosis or minimal changes on biopsy without an acute state of withdrawal (1/13). All patients with severe liver disease unrelated to alcohol (3/3) had marked elevations in plasma proline (Tables 1 and 2).

In the baboon model of alcohol feeding, venous plasma proline was significantly depressed in the alcohol-fed baboons compared to the pair-fed controls (216 ± 21 vs. 355 ± 35 μM; $p < 0.01$). Proline levels were not remarkably different between those animals with cirrhosis (n = 2; proline = 142,331) or fibrosis (perivenular and perisinusoidal) (n = 1; proline = 248) and those animals with simple fatty liver (n = 6; $\bar{x}$ = 184.7 ± 27.0).

The five pairs of baboons that were catheterized included two alcoholic-fed baboons with steatosis plus perivenular fibrosis, two with steatosis plus perisinusoidal and perivenular fibrosis, and one with transition to cirrhosis. The mean arterial blood alcohol in the alcohol-fed baboons was 21.0 ± 4.6 mM at the time of sampling. No alcohol was measurable in the blood of the pair-fed controls. The arterial plasma proline levels were not significantly different in the fasting state in the alcohol-fed animals compared to the controls (205 ± 35 vs. 185 ± 28), but arterial blood lactate was significantly increased in the alcohol-fed animals (6.17 ± 1.75 vs. 1.59 ± 0.14 mM, $p < 0.05$). Both in the alcohol-fed animals given the alcohol-containing diet and in the controls given the control diet, arterial proline levels tended to decrease at 1 to 2 hr (alcohol fed, 135 ± 19; control, 157 ± 20). Hepatic venous proline also was not significantly different between alcohol-fed animals and controls following an overnight fast (172 ± 32 vs. 131 ± 16). Hepatic vein lactate showed a trend to increased values in the alcohol-fed animals (2.15 ± 0.41 vs. 1.04 ± 0.21; not significant). Hepatic vein proline tended to fall in the alcohol-fed animals postprandially (159 ± 25) and tended to increase slightly in the control animals (157 ± 14) although none of these changes attained significance. In none of the samples of arterial or hepatic venous blood in the alcohol-fed animals did the proline exceed 352 μM.

Blood lactate was measured in 65 of the subjects at the time of plasma proline analysis. All controls studied had values less than 1.3 mM, the upper limit of normal designated in the Sigma Chemical Co. kit (17). All patients with severe liver disease studied (alcoholic and nonalcoholic) had elevated values (Table 1). As anticipated, 7/9 alcoholics with acute alcohol-related withdrawal symptoms and alcohol present in the blood had elevated values, but after more prolonged withdrawal (up to 72 hr and no alcohol in the blood) only 1/9 had such elevations. Among the patients with cirrhosis (not severe) 5/11 had elevated blood lactate values. Eleven of the alcoholics with cirrhosis had histological evidence of "active cirrhosis" with inflammation and/or alcoholic hepatitis in addition to cirrhosis. The mean proline value for this group of 11 was 257 ± 34 μM, which was not significantly different from the mean value for the group of patients with moderate cirrhosis (225 ± 18).

Among the alcoholics, there was a statistically significant positive correlation between plasma proline and blood lactate (n = 49, r = 0.766, $p < 0.001$) (Figure 1) as well as among the nonalcoholics with liver disease (n = 6, r = 0.8727, $p < 0.01$). There was no significant correlation between proline and lactate when the alcoholics with normal and elevated proline values were analyzed separately. Among the nonalcoholics plasma proline was also significantly correlated with elevation of the prothrombin time (n = 22, r = 0.6824, $p < 0.001$) and negatively correlated with serum albumin (n = 22, r = −0.7122, $p < 0.001$). Among the alcoholics there was a statistically significant positive correlation between blood lactate and elevation of the prothrombin time (n = 49, r = 0.4768, $p < 0.001$) but not between plasma proline and elevation of the prothrombin time or depression of serum albumin. There were no significant correlations between plasma proline or blood lactate and elevations of serum bilirubin.

Among all patients with normal plasma proline levels, depressed serum albumin was present in only 14%, prothrombin time elevations were present in 13%, and serum bilirubin was increased in 24%. By contrast among patients with elevated proline levels, serum albumin was decreased in 37%, the prothrombin time was elevated in 42%, and the serum bilirubin was elevated in 52%.

DISCUSSION

The results of this study demonstrate that hyperprolinemia occurs in only a minority of patients with alcoholic liver disease, including those with cirrhosis, if they

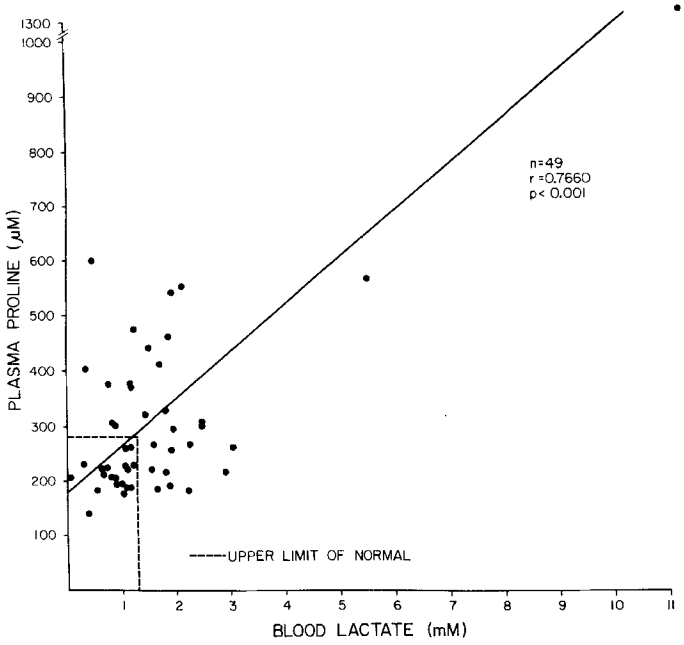

FIG. 1. Relationship between plasma proline and blood lactate in alcoholics. There was a statistically significant positive correlation between plasma proline and blood lactate over the range of proline values studied. A similar positive correlation was observed in patients with liver disease unrelated to alcohol.

are withdrawn from alcohol. Furthermore, patients with liver disease unrelated to alcohol may have marked elevations in plasma proline if the disease is severe. In patients with alcoholic liver injury characterized by minimal inflammation, hyperprolinemia is rare; in the baboon model of alcoholic liver injury in which cirrhosis develops in the absence of marked inflammation and necrosis, plasma proline is in fact depressed. Hyperlactacidemia rarely occurs in the alcoholic if alcohol has been withdrawn for 72 hr unless severe liver disease is present.

Fulminant liver injury of viral etiology is known to cause a generalized increase of plasma amino acids including proline (5). Thus, hyperprolinemia is not a unique feature of alcoholic liver injury. On the other hand, it is not surprising that patients with alcoholic hepatitis (including inflammation and necrosis on biopsy) may have hyperprolinemia. In the patients we studied, hyperprolinemia was observed only rarely in those with fatty liver (including perivenular fibrosis) or cirrhosis on liver biopsy. In comparable patients with fatty liver and perivenular fibrosis, follow-up studies have revealed a progression of liver injury (including the development of cirrhosis) over a period of a few years in a majority of those that continued to drink (20). Thus, a high plasma proline does not appear to be a useful marker of fibrogenesis in such patients. It is noteworthy, however, that in actively drinking alcoholics in whom alcohol is present in the blood at the time of study, both proline and lactate are frequently elevated. It is therefore possible that in some alcoholics, hyperprolinemia could promote fibrogenesis during drinking. Recent evidence, however, has shown that in rats a high hepatic proline concentration did not induce collagen synthesis (21).

In the primate model of alcohol feeding, cirrhosis can develop without a phase of "alcoholic hepatitis" (19), hyperprolinemia is absent and, in fact, plasma proline is depressed. Furthermore, in this model we have demonstrated that fibrogenesis is a continuous and active process in a majority of animals. Indeed, at the fatty liver stage, animals on diet for 7 months were found to have a 204% increase in collagen content compared to pair-fed controls as well as a significant increase in hepatic peptidyl proline hydroxylase activity (22). The activity of this enzyme was also increased when measured in animals treated for 14 months. These data and the previously published histological progression in a majority of animals (19) suggest that fibrogenesis is an active and continuous process in these animals despite the absence of elevated plasma proline levels.

An increase in blood lactate is consistently observed after administration of alcohol and has been attributed to the increased NADH:NAD ratio in the liver produced during the metabolism of alcohol by alcohol dehydrogenase (23). The rise in lactate, however, is transient and lasts for only a brief period of time following cessation of drinking as does the increased NADH:NAD ratio. Both changes are gone 72 hr after cessation of drinking. Thus, the hyperlactacidemia reported by others 72 hr after alcohol (2) cannot be due to an effect of alcohol but rather must represent liver injury. Indeed, among our own patients hyperlactacidemia occurred commonly in patients with severe disease.

The correlation between hyperprolinemia and hyperlactacidemia that we observed was due primarily to the elevated values of proline and lactate in those patients with severe liver injury. In our alcoholics no significant correlation was observed between proline and lactate within the subpopulations of those with values within the normal range and those with values exceeding the normal range. Furthermore, the populations with high proline and lactate values also had markedly abnormal prothrombin times and depressed serum albumin levels. Thus, both variables studied may be merely associated with severity of liver injury rather than causally related. In lactic acidosis unrelated to liver disease, hyperprolinemia is observed but elevations of lactate reported in such patients greatly exceed the values seen in our patients with terminal liver disease (7). Thus, any extrapolations to patients with much less severe metabolic derangements must be interpreted with caution. In the baboon, despite the higher lactate levels, plasma proline was not increased in the alcohol-fed animals compared to controls. Thus, in this model there was no apparent causal relationship between blood lactate and proline values. Furthermore, a majority of the animals showed progressive hepatic fibrosis despite the absence of hyperprolinemia in either peripheral venous, arterial, or hepatic vein blood both in the withdrawal state and in the presence of ethanol.

The studies demonstrating increased proline values in alcohol-induced cirrhosis were performed on serum (1, 2) while other studies were performed on plasma (5–13). However, plasma and serum values for proline have been shown to be comparable (24); therefore this difference in methodology could not explain the differences in findings.

We conclude that hyperprolinemia occurs only infrequently in patients with alcoholic liver disease after withdrawal from alcohol. When present under these conditions it may indicate severity of liver injury rather than fibrogenesis, and in this regard it is not specific and may occur in liver disease of various etiologies.

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