Decreased Activity of Carnosinase in Serum of Patients with Chronic Liver Disorders

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We measured the activity of carnosinase, a prominent hepatic peptidase, in sera from 69 patients with liver disorders. Mean values (and SDs) for those with liver cirrhosis (17 cases) and hepatoma (seven cases) were 0.51 (0.28) and 0.68 (0.21) μmol/mL per hour, respectively—clearly less than for normal adults: 4.19 (0.95) μmol/mL per hour. Samples from 17 cases of chronic hepatitis also showed moderately decreased activity, 1.41 (0.97) μmol/mL per hour. In contrast, 14 cases of acute hepatitis generally showed values falling within the normal limits: 3.41 (1.97) μmol/mL per hour. Our results for carnosinase correlated with those for cholinesterase (r = 0.70) and with the concentration of albumin in serum (r = 0.59), but not with the activity of either creatine kinase, aspartate aminotransferase, or alanine aminotransferase in serum. Carnosinase values differed more among groups of disorders than did the values for cholinesterase or albumin. Measurement of serum carnosinase activity may be of clinical value in assessing the severity of chronic liver-cell damage, but not in differentiating liver disease from nutritional, muscle, or endocrine disorders.

Additional Keyphrases: carnosine • cholinesterase activity • other liver-function tests compared • fluorimetry

Acute liver-cell damage causes leakage of hepatic enzymes such as aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2), and lactate dehydrogenase (EC 1.1.1.27) into the circulation. When hepatic cells are chronically inflamed and become replaced by fibrous tissue, liver function progressively declines, especially the hepatic synthesis of protein. Such chronic liver dysfunction results in a decreased albumin concentration and decreased activity of cholinesterase (EC 3.1.1.8), among other components of serum (1). Thus, measurements of the concentration of serum proteins and of the activities of hepatic enzymes provide diagnostic clues to the extent of liver-cell damage (2). The diagnostic specificity of these test results, in terms of how well the values are separated according to the type of hepatic disorder, is not satisfactory, depending as it does on the nature and stage of a given pathological condition. Therefore, further clarification of the biochemical changes involved in such liver disorders is necessary to improve the specificity of laboratory diagnosis and facilitate the monitoring of such disorders.

We have been studying serum carnosinase (aminoacylhistidine dipeptidase, EC 3.4.13.3)—an enzyme richly distributed in the liver, kidney, and spleen (3, 4)—in various pathological conditions (5, 6). Its physiological role is not well known, but it hydrolyzes its dipetide substrate, carnosine, into its constituent amino acids (7). Examination of serum carnosinase activity in liver disorders, however, has not been reported so far, despite its predominant distribution in the liver. Thus we were interested in doing so, to see if the measurement has any clinical value in their differential diagnosis.

Materials and Methods

Subjects: Serum was sampled from 69 patients with liver diseases for which the clinical diagnosis was well established. They consisted of 31 men and 38 women, 35 to 81 years old. Their diagnoses and the number of cases were as follows: acute hepatitis, 13; chronic hepatitis, 17; liver cirrhosis, 17; hepatoma, seven; and fatty liver, three. The subjects used for determination of the normal reference interval, as reported previously (8), corresponded to the patients in terms of age and sex.

Measurement of enzyme activity: The method used in measuring carnosinase activity, described elsewhere (8, 9), can be summarized as follows. Dilute 150 μL of serum eightfold with Tris HCl buffer (50 mmol/L, pH 8.4). Mix 400 μL of the diluted serum with either 100 μL of 50 mmol/L carnosine or with 100 μL of the Tris buffer (for the control). Incubate the mixture for 1 hr, then terminate the reaction by adding 500 μL of 0.6 mmol/L trichloroacetic acid. Centrifuge and assay 100 μL of the supernate for 15 min with 2 mL of 0.3 mol/L NaOH and 400 μL of 75 mmol/L o-phthalaldehyde. Add 400 μL of 6 mol/L HCl and measure the fluorescence (emission wavelength 452 nm, excitation wavelength 344 nm).

In an automated chemical analyzer we measured the activity of aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase by the international standard methods recommended by the German Society of Clinical Chemistry. Serum albumin was determined by the bromcresol green dye-binding method, in an automated analyzer. We measured the activity of cholinesterase manually by a method in which benzoyl choline is the substrate, using a commercial kit supplied by Katayama Chemical Co., Osaka.

Results

Measured activities of serum carnosinase and cholinesterase, and the concentration of serum albumin, are shown in Figure 1 for the patients, grouped according to liver disorder. With regard to carnosinase, samples from patients with liver cirrhosis (0.51 ± 0.28 μmol/mL per hour) and hepatoma (0.68 ± 0.21 μmol/mL per hour) invariably showed much less activity than normal, the normal limits being 4.19 ±
0.95 μmol/mL per hour (means ± SD). Patients with chronic hepatitis also showed slightly to severely decreased activity (1.41 ± 0.97 μmol/mL per hour). In contrast, samples from cases of acute hepatitis (3.41 ± 1.09 μmol/mL per hour) generally had values falling within the normal limits, with only few samples exceeding those limits. Cases of fatty liver appeared to have slightly subnormal values, although the difference was not statistically significant.

The distribution of test results for cholinesterase and albumin among the groups of disorders showed a tendency similar to that of the carnosinase activity. As far as chronic liver disorders are concerned, the separation of the values among the different groups, in terms of percentage of samples with below-normal results, was more conspicuous in the case of carnosinase measurement than in the other two: the percentage of samples with below-normal carnosinase activity was 100% in liver cirrhosis, 88% in chronic hepatitis, and 100% in hepatoma; the corresponding percentages for these respective disorders for serum albumin measurement were 54%, 47%, and 50%.

We examined the interrelationships between the activity of carnosinase in serum and results of commonly used liver-function tests. The activity of carnosinase (y) showed (Figure 2) a statistically significant positive correlation with both cholinesterase (x) \( r = 0.70; y = -1.4 + 0.002x; p <0.01 \) and serum albumin \( r = 0.59; y = -6.5 + 2.33x; p <0.02 \), but no significant correlation with either creatine kinase, lactate dehydrogenase, or aspartate aminotransferase.

**Discussion**

The clinical significance of measuring serum carnosinase activity was first described in the case of hereditary deficiency of carnosinase with extensive neuromuscular involvement (10–13). Its significance in other disorders has not been well documented. We were intrigued by the fact that its substrate, carnosine, is widely distributed in skeletal muscle (14, 15). Thus we measured the activity of the enzyme in serum of patients with various neuromuscular disorders, finding it to be very low in the progressive stage of muscular dystrophy (6) and in hypothyroidism (16).

The current finding of low carnosinase activity in serum samples in cases of chronic liver disorders, especially those of liver cirrhosis and hepatoma, is not unexpected when one considers the fact that the enzyme is mainly produced by the liver (3). The discriminating power of the carnosinase measurement among different hepatic disorders appeared to be better than that for other conventional analytes such as cholinesterase, aspartate aminotransferase, alanine aminotransferase, or albumin. The diagnostic specificity of the measurement as a liver-function test, however, may not be high because we know that the activity also is altered in the above-mentioned extra-hepatic conditions.

The physiological mechanism involved in the decreased activity is unknown. A similar decrease in the enzyme activity in chronic liver diseases has been recently described for angiotensin-converting enzyme (17) and for a monooxygenase of thyroxin (18–20). Those enzymes were also found to be decreased in extra-hepatic conditions causing generalized malnutrition. Thus the decreased carnosinase activity we observed here may simply reflect the nutritional status of
the patient, proportional to the severity of the hepatic
disorder.

Nevertheless, our findings are of practical importance in
the differential diagnosis of disorders causing carnosinase
deficiency, such as carnosinemia, for which the measure-
ment was originally developed, and also as a supplement to
conventional liver-function tests, to enhance diagnostic ac-
curacy.

We thank Prof. Nanaya Tamaki, Department of Nutrition,
Kobegakui University, for his encouragement and useful sugges-
tions in this study. We are also grateful to Drs. Masaki Naka and
Nobuyuki Kasahara, Department of Pharmacy, Minoo City Hospi-
tal, for their generous support for the fluorimetry of the carnosinase
assay.

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