Plasma Amino Acid Abnormalities in Liver Disease : Comparative Analysis of Idiopathic Portal Hypertension, Extrahepatic Portal Occlusion and Liver Cirrhosis

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OUCHI, K., MATSUBARA, S., FUKUHARA, K. and MATSUNO, S. *Plasma Amino Acid Abnormalities in Liver Disease : Comparative Analysis of Idiopathic Portal Hypertension, Extrahepatic Portal Occlusion and Liver Cirrhosis.* Tohoku J. Exp. Med., 1989, **158** (2), 171-178 — To clarify the clinical significance of specific plasma amino acid abnormalities occurring in liver disorders with portal-systemic shunting, plasma amino acids and insulin levels were measured in idiopathic portal hypertension (IPH), extrahepatic portal occlusion (EHPO), and liver cirrhosis (LC). Three branched chain amino acids (BCAA : valine + leucine + isoleucine) were decreased in all three diseases in comparison with controls. Since plasma insulin measured during oral glucose tolerance tests did not specifically rise in LC, reduction of BCAA is not merely ascribed to hyperinsulinemia. Either portal-systemic shunting or some extent of liver damage may contribute to a fall in BCAA. Two aromatic amino acids (AAA : phenylalanine + tyrosine), which were within the normal range in EHPO and IPH, showed a marked increase in LC. Thus, changes of AAA probably mainly reflect the severity of the liver disease. The molar ratio of BCAA/AAA (MR) significantly correlated with ICG k, ICG R₁₅, PT and the sum of blood ammonia in an oral ammonia tolerance test which may reflect the degree of hepatic disorder. MR diminished in the following decreasing order : controls, EHPO, IPH and LC. ——— amino acid analysis ; IRI ; idiopathic portal hypertension ; extrahepatic portal occlusion ; liver cirrhosis

The abnormal plasma amino acid pattern of liver cirrhosis (LC) is characterized by an increase of two aromatic amino acids (AAA : tyrosine + phenylalanine), methionine and tryptophan, and a reduction of three branched-chain amino acids (BCAA : valine + leucine + isoleucine). The molar ratio (MR) of BCAA/AAA in LC is, therefore, decreased. In addition, a decrease of MR is more predominant in patients with hepatic encephalopathy, mainly due to a marked decrease of BCAA (Chase et al. 1978). Different amino acid patterns can

be detected in various kinds of hepatic diseases, and these differences are influenced by the severity of the hepatic disease and/or the presence of portal-systemic shunting. However, the contribution of hepatocellular damage and portal-systemic shunting to these changes in plasma amino acid concentrations remained unknown. Decreased plasma levels of BCAA in patients with LC could be ascribed to hyperinsulinemia (Munro et al. 1975; Soeters and Fischer 1976). In addition, some investigators attributed hyperinsulinemia, that is present in LC, to hepatic parenchymal damage (Holdsworth et al. 1977; Smith-Laving et al. 1979), and others to portal-systemic shunting (Shurberg et al. 1977; Iwasaki et al. 1980). This study was undertaken to assess the contribution of hepatic damage or portal-systemic shunting to the abnormalities of plasma amino acids that occur in patients with extrahepatic portal occlusion (EHPO) and idiopathic portal hypertension (IPH), who have normal or almost normal liver function tests and minimal or minor histological abnormalities of the liver, in comparison with LC.

PATIENTS AND METHODS

Plasma amino acid levels were measured in 25 patients with IPH, 43 with LC and 6 with EHPO in their preoperative periods. Plasma amino acid levels were also measured in the postoperative or follow-up periods in some patients with IPH and LC. All patients studied had marked esophageal varices without any sign of hepatic encephalopathy, and they were subjected to the Sugiura's procedure which consists of esophageal transection and proximal gastric devascularization (Sugiura and Futagawa 1973). Ten healthy adults were used as controls. Diagnosis was made not only by histologic examination of the liver, but by abdominal angiography and hepatic venography.

Measurement of free amino acid levels in blood was performed using fasting blood collected early in the morning with heparinized syringes, plasma was deproteinized with 5% sulfosalicylic acid. After centrifugation at 2,500 rpm for 10 min, the respective amino acid concentrations of the supernatant were measured by an amino acid autoanalyzer (JEOL 8 AH, Tokyo). The aminogram of patients with liver diseases was expressed as percent changes of the normal control. Plasma immunoreactive insulin (IRI) levels were measured every 30 min for 2 hr during a 75 g oral glucose tolerance test (OGTT). Insulin was measured by a double antibody RIA method. Correlations of the MR values with the disappearance rate of 0.5 mg/kg b.w. of indocyanine green (ICG k), plasma retention rate of indocyanine green (ICG R_{15}), prothrombin time (PT) and the sum of blood ammonia levels at 0, 30, 60, 90 and 120 min in an oral ammonia tolerance test (50 mg/kg of ammonium chloride, ΣNH_3) were also evaluated in LC and IPH. EHPO was not included because of a small number of patients.

The results of the amino acids and IRI levels in plasma were evaluated using the analysis of variance by Kruskal Wallis followed by the Kruskal Wallis test for multiple comparisons modified with the Bonferroni method (Wallenstein et al. 1980). A *p* value less than 0.05 was considered to be significantly different.

RESULTS

Changes of the amino acid pattern in IPH, EHPO and LC were basically similar among the 3 groups (Fig. 1). In particular, between IPH and EHPO, no significant difference was found in all amino acid levels except for a significant reduction of leucine in IPH. Levels of valine, leucine and isoleucine were

Fig. 1. Plasma amino acid patterns in idiopathic portal hypertension (IPH), extrahepatic portal occlusion (EHPO) and liver cirrhosis (LC). $\circ$, IPH ($n = 25$); $\bullet$, EHPO ($n = 6$); $\triangle$, LC ($n = 43$). ** $p < 0.01$ compared with IPH and EHPO; $\dagger p < 0.05$ compared with IPH.

decreased in the 3 diseases, and no significant difference was noted between IPH and LC. On the other hand, glutamic acid was markedly increased in all 3 diseases. Phenylalanine and tyrosine, which were within the control range in IPH and EHPO, were markedly increased in LC.

As shown in Fig. 2, BCAA was significantly low in the 3 diseases compared with the normal control. Although there was no difference between IPH and

Fig. 2. Values of branched-chain amino acids (BCAA: Val+Leu+Ile), aromatic amino acids (AAA: Tyr+Phe) and molar ratio (MR) of BCAA to AAA in idiopathic portal hypertension (IPH), extrahepatic portal occlusion (EHPO) and liver cirrhosis (LC). Open columns indicate normal control. Values are mean $\pm$ S.D. a, $p < 0.01$ compared with normal control; b, $p < 0.05$ compared with IPH; c, $p < 0.01$ compared with normal control, EHPO and IPH; d, $p < 0.01$ compared with normal control; e, $p < 0.01$ compared with normal control; f, $p < 0.05$ compared with EHPO.

Fig. 3. Plasma IRI levels during a 75 g oral glucose tolerance test in idiopathic portal hypertension (○, $n=9$), extrahepatic portal occlusion (●, $n=5$) and liver cirrhosis (△, $n=7$). Values are means $\pm$ s.d. There were no significant differences in IRI levels among three groups.

EHPO, BCAA was significantly higher in LC than in IPH. On the other hand, no difference of AAA was found among the normal group, IPH and EHPO, but a significant increase was found in LC as compared with IPH and EHPO. MR was significantly low in the 3 diseases compared with the normal control. Although MR tended to diminish in the following decreasing order: EHPO, IPH and LC, there was not significant difference between IPH and LC. However, MR in LC was significantly lower than that in EHPO. There were no statistically

Fig. 4. Serial changes in molar ratio (MR) of BCAA to AAA in liver cirrhosis before and after the Sugiura's procedure.

significant differences in IRI levels throughout OGTT among patients with IPH, EHPO and LC (Fig. 3). In patients with LC, IRI levels ranged with a great variance at 90 and 120 min after glucose administration.

With respect to the MR changes in LC before and after the Sugiura's procedure (Fig. 4), a sudden fall of MR was noted in those who died of hepatic

Fig. 5. Serial changes in molar ratio (MR) of BCAA to AAA in idiopathic portal hypertension before and after the Sugiura's procedure.

Fig. 6. Correlations between molar ratio (MR) of BCAA to AAA and ICG K, ICG R_{15} , prothrombin time (PT) and the sum of blood ammonia levels in an oral ammonia tolerance test (ΣNH_3) in idiopathic portal hypertension (IPH) and liver cirrhosis (LC).

insufficiency in the immediate and late postoperative periods. In addition, MR was reduced gradually in some cases during the follow-up period. On the other hand, most patients with IPH showed no remarkable changes of MR in the post-operative and follow up periods (Fig. 5).

MR values were significantly correlated with ICG k, ICG R₁₅, PT and ΣNH_3 in LC and IPH (Fig. 6).

DISCUSSION

Idiopathic portal hypertension (IPH) is a clinical disorder of unknown etiology, characterized by splenomegaly, pancytopenia, and portal hypertension of unknown cause with no evidence of LC and extrahepatic portal occlusion (EHPO). Although the liver weight in the IPH shows a slight atrophic tendency, the ratio of the liver weight to the body weight is not significantly lower than normal. Marked narrowing of portal vein branches is assumed to be one of the essential changes of the liver in IPH (Aida et al. 1982). Almost normal histological findings are obtained in most of the cases with EHPO. Peripheral portal obstruction has also been noted in EHPO, though in a milder degree than IPH (Arakawa et al. 1982). In both IPH and EHPO, liver function is usually well maintained. In the surgical treatment of esophageal varices, operative mortality and follow-up results are, therefore, strikingly different among patients with IPH, EHPO and LC. Of 48 patients with IPH who were subjected to the Sugiura's procedure in our department, two died during the 30-day postoperative period of cerebral hemorrhage and bleeding from gastric varices after emergency esophageal transection. Other three patients died during the follow-up period from unrelated causes (traffic accident, rectal cancer and an unknown cause). Of 14 patients with EHPO, none died in the 30-day postoperative period and one died of burns in the follow-up period. None of the patients with IPH and EHPO died of progressive hepatic insufficiency. Of 128 patients with LC, in contrast, 11 died within the 30-day postoperative period and 52 died during the follow-up period, 38 from progressive liver failure.

Reduction of BCAA was found in IPH, EHPO and LC. There are some studies reporting a relationship between reduction of BCAA and hyperinsulinemia in LC and liver insufficiency (Munro et al. 1975; Soeters and Fischer 1976). Hyperinsulinemia, probably caused from deteriorated insulin metabolism in the liver, reduced the blood BCAA levels due to an accelerated consumption in the muscle and the fat tissue (Johnston et al. 1977). On the other hand, there are also many reports which deny a relationship between reduction of BCAA and hyperinsulinemia in LC (Iwasaki et al. 1980; Marchesini et al. 1980, 1983). Iwasaki et al. (1980), and Sherwin et al. (1974) reported that the reduction of BCAA in LC was mainly attributable to portal-systemic shunting, since hyperinsulinemia, found in LC, has not been associated with EHPO (Smith-Laving et al. 1979) and IPH (Iwasaki et al. 1980). Our results partially support this concept, since there

was no significant difference in IRI levels throughout OGTT among IPH, EHPO and LC, and there was a more marked reduction of BCAA in IPH than in LC. These findings indicate that the decrease of BCAA does not reflect the severity of parenchymal liver damage. However, even in patients with little liver damage as exemplified by EHPO and IPH, a fall in BCAA can be found mainly due to increased peripheral utilization of these amino acids. This suggests that BCAA is nonspecific nitrogen carrier to the periphery, where its fate may be either protein synthesis or transamination. Morgan et al. (1978) suggested that a decrease of gluconeogenesis in the liver is an early consequence of liver damage. Further studies referring to the effect of portal-systemic shunting or liver dysfunction regardless of its severity on the reduction of plasma BCAA are still required to clarify this point.

AAA was within the normal range in IPH and EHPO, and significantly increased in LC. This evidence suggests that portal-systemic shunting is not directly related with the increase of AAA. It has been reported that increase of AAA correlates well with the degree of disturbance in liver parenchyma (Morgan et al. 1978), and not only accelerated catabolism of muscle protein but also an impaired extraction by the damaged cirrhotic liver is considered to be one of the causes of this alteration.

Both markedly increased levels of AAA and reduced levels of BCAA contributed to the excessive reduction of MR in patients with LC. On the other hand, in patients with EHPO and IPH, MR was slightly, but significantly decreased in comparison with controls, but AAA did not differ from controls.

MR value appeared to reflect the severity of the liver disease. Morgan et al. (1978) reported that a good correlation existed between MR values and the severity of the liver disease as judged histologically. We also found significant correlation between MR values and various biochemical parameters such as ICG k, ICG R₁₅, PT and ΣNH_3 , all of which may be applicable indicators to measure liver cell function. Koyama et al. (1981) reported that ΣNH_3 may reflect the degree of hepatic parenchymal disorders rather than the hepatic circulatory abnormalities. Sudden or gradual reduction of MR after operative procedures in LC might indicate aggravation of liver dysfunction. On the other hand, the MR value showed no remarkable changes in IPH during the postoperative and follow-up periods, which is considered to be related to the fact that none of patients with IPH died of progressive hepatic insufficiency.

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