HEPATIC GLUCONEOGENIC KEY ENZYMES IN PATIENTS WITH HEPATIC CANCER

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Summary

Key enzymes of gluconeogenesis in the liver, phosphoenolpyruvate carboxykinase [EC 4.1.1.32] and glucose-6-phosphatase [EC 3.1.3.9], were studied in patients with primary or metastatic hepatic cancer. Liver specimens for enzyme assay were obtained by necropsy performed within four hours after death. It was confirmed that both enzyme activities in rat liver preserved at 4°C remained unchanged within nine hours after the removal of the tissue. Activities of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase decreased to below ten per cent of the control in neoplastic liver tissue of patients with hepatocellular carcinoma accompanied with liver cirrhosis. These two enzyme activities in cirrhotic tissue of patients with hepatocellular carcinoma were lower than those in patients merely with cirrhosis. In patients with metastatic hepatic cancer both two enzyme activities further decreased and were scarcely detected not only in neoplastic tissue but also in non-neoplastic tissue. These results show that hepatic gluconeogenesis markedly decreases in patients with primary or metastatic hepatic cancer. The biochemical analysis of the blood in hepatic cancer, decreased in blood glucose and release in immunoreactive glucagon, also suggested the suppression of gluconeogenesis.

Key Words: hepatic cancer, gluconeogenic key enzymes, glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, immunoreactive glucagon.

Introduction

Phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) are key enzymes of gluconeogenesis in the liver. PEPCK catalyzes to carboxylation of phosphoenolpyruvate and the activity of this enzyme is the lowest among the gluconeogenic key enzymes. Therefore, changes of this enzyme activity reflect the activity of hepatic gluconeogenesis. Recently, Hommes et al. and Vidnes et al. demonstrated cases of PEPCK deficiency in the liver and showed that persistent neonatal hypoglycemia was caused by deficiency of PEPCK.

The association of hypoglycemia with hepatocellular carcinoma, so-called paraneoplastic syndrome, is well-known. There are several reports upon activities of hepatic glycolytic key enzymes and G-6-Pase in patients having hepatocellular carcinoma associated with hypoglycemia. Taketa et al. showed that activity of G-6-Pase in neoplastic tissue was reduced in the same degree as in cirrhotic tissue.
McFadzean et al.\textsuperscript{5} showed that G-6-Pase activity markedly decreased in neoplastic liver tissue and that the decrease enhanced hypoglycemia by cooperating with increased glycolysis. G-6-Pase plays an important role in hepatic gluconeogenesis as well as in glycolysis, but so far its activity has not yet been estimated in patients with hepatocellular carcinoma without incidence of hypoglycemia. Hammond et al.\textsuperscript{9} reported that activities of gluconeogenic key enzymes including PEPCK were considerably reduced in the tissue of hepatocellular carcinoma. Up to now these enzyme activities in primary hepatic cancer have not yet been compared with those in metastatic hepatic cancer; furthermore, little attention has been paid to differences of these enzyme activities between neoplastic and non-neoplastic tissue.

Liver specimens were mainly obtained from patients with hepatic cancer within four hours after death. The effect of temperature and time elapsed after the excision was examined on these two enzyme activities in rat liver, and then they were measured in patients with primary or metastatic hepatic cancer. This paper deals with PEPCK and G-6-Pase activities in patients with primary or metastatic hepatic cancer and on the relationship between these two enzyme activities and pancreatic hormones which regulate activities of key enzymes of carbohydrate metabolism.

**Materials and Methods**

**Animals:** Mature male Wistar strain rats, weighing about 400–450 g, were used. Animals had free access to food and water.

**Enzyme assays:** PEPCK activity was determined by modification of the method of Seubert and Huth\textsuperscript{10,11}. Liver homogenates were prepared as follows; Rat and human liver were homogenized in a Potter-Elvehjem homogenizer, in 50 mM tris-HCl buffer containing 1 mM dithiothreitol at pH 7.4. A 105,000\times g supernatant was taken for enzyme assay and protein determination. Enzymes were prepared at 2\textdegree{}C to 4\textdegree{}C. One unit of enzyme was defined as the amount catalyzing the formation of one \textmu{}mole of phosphoenolpyruvate per minute under the standard assay condition. G-6-Pase was assayed by the method of Swanson\textsuperscript{12}. Protein was determined by the method of Itzhaki and Gill with bovine serum albumin as a standard\textsuperscript{13}.

**Chemicals:** Lactate dehydrogenase [EC 1.1.1.27] from rabbit muscle, pyruvate kinase [EC 2.7.1.40] from rabbit muscle and dithiothreitol were purchased from Boehringer-Mannheim Japan Co., Tokyo. Oxaloacetic acid, NADH, ADP and glucose-6-phosphate were products of Sigma Chemical Co. St., Loui. Antibody for glucagon, 30K was purchased from Hoechst Japan Co., Tokyo. Other chemicals were standard commercial products.

**Subjects:** Activities of G-6-Pase and PEPCK were assayed in five patients with hepatocellular carcinoma, one with hepatocholangioma, three with metastatic hepatic cancer, two with chronic liver diseases and three controls without liver diseases. All cases of hepatocellular carcinoma were accompanied with liver cirrhosis. Specimens of liver were obtained from patients with primary or metastatic hepatic cancer by necropsy. The remains were kept at 4\textdegree{}C and necropsy was performed within four hours after death. Both in other liver diseases and in controls wedge biopsy specimens of liver were taken at laparotomy with patients' consent. For biochemical analysis of the blood, fifteen patients with hepatoceUular carcinoma and eight patients with metastatic hepatic cancer were employed.

**Biochemical analysis of the blood:** Serum and plasma were prepared from venous blood of three patients at fasting. Blood glucose was assayed with glucose-oxidase\textsuperscript{14}. Serum insulin\textsuperscript{15} and C-peptide\textsuperscript{16} were measured by