A new surgical procedure consisting of ligation of the common hepatic artery and auto-transplantation of hepatocytes into the spleen for end stage liver cirrhosis accompanied by ascites

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Summary: The authors developed a new surgical procedure for end stage liver cirrhosis associated with ascites. This procedure consists of ligation of the common hepatic artery and hepatocyte inoculation into the spleen (method A) and in this study is compared with common hepatic artery ligation alone (method B). Six of the eleven dogs operated by method A survived for six months or more with a significant (P<0.01) difference in the three month survival in comparison with method B. In the hemodynamic study of both methods, the portal vein pressure and portal resistance decreased as a result of operation, but in method B, they returned to preoperative levels and in method A the low levels persisted for more than one year. In our method, liver function improved remarkably after three months. The heparplastin and the cholinesterase levels increased after three months in method A with a significant difference (P<0.01) in comparison with method B. The labeling index (L.I.) of intrasplenic hepatocytes also increased three months later. We emphasize that our method is an ideal procedure not only to improve portal haemodynamics but also to improve liver function, in end stage cirrhosis. Gastroenterol Jpn 1993;28:259-267.

Key words: end stage cirrhosis; hepatic artery; autotransplantation hepatocytes.

Introduction

Ligation of the common hepatic artery, gastroduodenal artery, right gastric artery and splenic artery was found to be effective in controlling intractable ascites due to liver cirrhosis by Rienhoff¹. Berman, Altemeier, and Honjo²-⁴ reported its effectiveness as well. Taylor, Janke and Deforges⁵-⁷ on the other hand showed that the procedure was ineffective. This procedure has not been used since 1955.

Mito⁸ transplanted hepatocytes into the spleen in five cases with cirrhosis, although the growth of the hepatocytes was limited to a short period. Transplantation of hepatocytes has been performed for acute hepatic failure by Sutherland⁹. There have been no reports on the transplantation of hepatocytes in chronic hepatic disease, except for those of Mito⁸ and Elias¹⁰.

Taking into consideration the likelihood of hepatic failure when Rienhoff's method is used, we ligated the common hepatic artery only to treat intractable ascites in liver cirrhosis. The ligation of only the common hepatic artery does not lead to sudden decrease of hepatic blood flow.

Considering preservation and improvement of liver function in liver cirrhosis to be as important as the control of ascites, therefore excised about 35% of autogenous liver and inoculated free hepatocytes into the spleen.

Experimental Methods

Production of liver cirrhosis and ascites in dogs:
Ninety-eight mongrel dogs weighing 10–20 kg were used, including ten normal dogs as controls. Two milliliters of carbon tetrachloride (CCL₄) per kilo were administered to 88 dogs through a stomach tube once a week for 4 weeks. Since no ascites developed by this method alone, half of the supradiaphragmatic inferior vena cava (IVC) was constricted 8 weeks after the administration of CCL₄. Twenty-five of the 88 dogs lived for 24 hours or more after constriction. Approximately 22 hours after constriction of the IVC, 500–1000 ml of ascitic fluid appeared. Three days after the constriction, 2 of the 25 dogs died of sepsis. The surviving dogs (n=23) were divided into two groups. One group underwent ligation of the common hepatic artery with hepatocyte inoculation into the spleen (method A, n=11). The other group underwent ligation of the common hepatic artery only (method B, n=12) (Figure 1).

Preparation of free hepatocytes
The right upper and lower lobes, the middle lobe and part of the caudate lobe were excised, comprising about 35% of the total weight of the liver. A cannula was inserted into the main portal vein of the excised liver for preliminary perfusion with a solution, heated to 37°C and containing 2 units of heparin per ml, 0.5 mM EGTA and Hank's solution (pH 7.5) without Ca. Then the excised liver was perfused with Ca²⁺ solution containing 0.05% collagenase, 0.10% hyaluronidase and Hank's solution (pH 7.5) without Mg²⁺ for 15 minutes. When the liver became white, it was cut into thin slices with ophthalmic scissors, and shaken at 37°C. The suspension was filtered through a 250 μm nylon mesh and the volume adjusted to 100 ml with suspension buffer, pH 7.6 at 37°C (4.0 g NaCl, 0.4 g KCl, 0.15 g KH₂PO₄, 0.1 g Na₂SO₄, 0.13 g MgCl₂·6H₂O, 0.18 g CaCl₂·2H₂O, 20 mM N-2-hydro-di-ethylpipera-zine-N-2-ethansulfonic acid (HEPES), and H₂O ad 1000 ml). The hepatocyte suspension was washed twice with cooled Hank's solution containing Ca²⁺ and Mg²⁺ and centrifuged at 300 rpm for three minutes. A higher final yield of cells can be obtained at the expense of lower purity by increasing the centrifugation force (600 rpm for 4 minutes)².

Viability of isolated hepatocytes was determined by counting the viable cells with isotonic 0.6% Trypan blue. The viability was about 72%. A suspension of 1 × 10¹⁰ hepatocytes was injected into the splenic parenchyma through a No. 18 gauge needle. The splenic hilum was clamped for about 20 minutes after the injection. All the dogs were given intravenous penicillin postoperatively for 5 days.

Determination of haemodynamics
For measurement of portal vein pressure (PVP) a polyethylene tube was inserted into the truncus of the portal vein through a branch of the superior mesenteric vein. This pressure was determined on a mercury manometer. For arterial pressure, a polyethylene tube was inserted into the femoral artery and the arterial pressure was determined with a mercury manometer. For the central vein pressure (CVP), a polyethylene tube was inserted into the saphenous vein and placed in the superior vena cava.

Probes of an electromagnetic flowmeter (Cardina Medical Electronics) were applied to the hepatic arteries and the truncus of the portal vein for determination of the hepatic arterial blood flow (HAF) and portal vein blood flow (PVF). The total HAF was calculated by adding the volume of each hepatic arterial blood flow. For the determination of PVF, the tip of the probe was placed where the splenic vein joins the portal vein truncus. Portal vein resistance (PVR) was obtained by the following formulas PVR=(PVP-CVP)/PVF [cm H₂O·min/ml].