Hepatic Oxygen Supply, Energy Charge, and Histological Findings in Dogs with Portal Vein Arterialization

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(Received 17 April 1997/Accepted 16 June 1997)

ABSTRACT. Hepatic oxygen supply, energy charge (EC), and histology were examined comparatively in dogs with portal vein anastomosis (PVA group), and PA in addition to PVA (PA group). The PVA group showed a lower level of hepatic oxygen supply than those of the PA group throughout the experimental period, and also showed decreases of adenosine triphosphate (ATP) and EC level after blood perfusion. In contrast, the oxygen supply and consumption were stable in the PA group. A temporary fall of ATP level was followed by recovery to the preperfusion level in the PA group. Histological examination indicated the collapse of hepatic cords with granular and vacuolar degeneration in only the PVA group. These findings suggested that PA, when supplemented to PVA, is an available technique for preventing hepatic failure caused by ischemic conditions. — KEY WORDS: canine, liver, portal arterialization.

The portal vein arterialization technique has been used to prevent hepatic failure associated with surgical portocaval shunting to reduce portal hypertension, which is commonly associated with chronic liver disease and cirrhosis [3, 17]. The portocaval shunting, called portal vein anastomosis (PVA), reduces total hepatic blood flow volume (THBF), which results in decreased oxygen supply to hepatic cells because the oxygen supply completely depends on THBF which consists of hepatic artery (HAF) and portal vein blood flow (PVF). It has been generally accepted that the most important factor for maintaining liver functions is oxygen supply to hepatic cells since ligation of the hepatic artery [5, 13] or portal vein [7, 10, 11] induces severe hepatic failure. Therefore, portal vein arterialization in addition to PVA (PA) is considered to be one available technique for preventing liver disfunctions associated with ischemic conditions caused by PVA and/or some hepatic disorders. There is, however, little information concerning hepatic energy metabolism in dogs with PA.

This paper deals with the hepatic oxygen supply and consumption, energy charge (EC), and morphological findings in dogs with PVA and PA.

Dogs: Seven clinically healthy dogs (mixed breed, 1–3 years old, weighing 10.5–16.0 kg) were kept over a week on a commercial diet and water ad libitum. After habituation, these dogs were randomly divided into PVA group (3 dogs) and PA group (4 dogs).

Anesthesia: After fasting for 24 hr, each dog was premedicated with glycopyrrolate (0.005 mg/kg, s.c.) to induce anesthesia with thiamylal sodium (25 mg/kg, i.v.). Intratracheal intubation was performed before maintaining anesthesia with nitrous oxide and oxygen (2:1 v/v) under the intravenous administration of 0.1% ketamine hydrochloride in normal saline at the rate of approximately 50 ml/kg/hr. The respiration was controlled by injection of suxamethonium chloride (0.2 mg/kg, i.v.) during the experimental period.

Portal vein arterialization and anastomosis: The operations for PA and PVA were carried out as illustrated in Fig. 1. The abdomen was medisected to perform splenectomy. After an intravenous injection of heparin sodium (100 U/kg), the portal vein on the mesenteric side was anastomosed to the right femoral vein for shunting portal venous blood into the right femoral vein with a pump.
K. YAMAZOE, ET AL.
(Microprocessor Pump Drive, Cole-Parmer Instrument Co., U.S.) (PVA). After construction of PVA, the operation for complete PA in combination with a ligation of the hepatic artery was performed. In short, the portal vein on the hepatic side was cannulated to perfuse arterial blood from both the femoral and carotid arteries with another pump. Then, about 1.5 l of supplementary heparinized (10 U/ml) arterial blood collected from other dogs was also perfused for controlling hepatic blood flow volume.

Experimental design: The blood flow rate of the hepatic artery and portal vein were determined by an electromagnetic flowmeter (MVF-3000, NIHON KOHDEN, Japan). For monitoring of the arterial and the hepatic venous pressure, 12 Fr. and 5 Fr. cathethers were respectively inserted into the left femoral artery and the hepatic vein via the right jugular vein. In both PA and PVA groups, the flow rate was maintained at more than 50% of the preclamping total hepatic blood flow volume (THBF: HAF + PVF). The time when blood flow was stabilized, was designated as preperfusion.

Samples: Blood samples were collected from the arterialized portal vein and hepatic vein in PA, and from the hepatic artery and vein in PVA at 0.5, 1, 2 and 3 hr after perfusion, respectively. At the same time, small pieces of liver tissue samples were obtained to measure adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) for determining EC.

Oxygen supply and consumption: From the data of blood gas analysis, hepatic oxygen supply (HO2 supply) and hepatic oxygen consumption (HO2 consumption) were calculated as follows [8]: HO2 supply (ml/min) = 1/100 x THBF (PVF in PA or HAF in PVA) x C (oxygen content of PVF or HAF) and HO2 consumption (ml/min) = 1/100 x THBF x [C-Chv (oxygen content of hepatic vein)].

Figure 2 shows the changes of the arterialized portal vein and hepatic artery pO2 in the PA and PVA groups, respectively and hepatic vein pO2 in both groups after perfusion. No significant differences in the arterialized portal vein and hepatic artery pO2 were detected between the two groups. Although no significant difference in preperfusion hepatic vein pO2 values was observed between the PA and PVA groups (59.4 ± 22.8 and 59.4 ± 35.4 mmHg, respectively), pO2 values in the PA group were significantly higher (p<0.01) than those in the PVA group at 1, 2 and 3 hr after perfusion, suggesting that the PA operation was able to produce a larger oxygen supply to hepatic cells via only PVF compared to that by HAF. The changes of hepatic oxygen supply and consumption after perfusion are shown in Fig. 3. Although the oxygen supply in both groups was kept stable after perfusion, the mean preperfusion value in the PA group was higher than that in
PORTAL ARTERIALIZATION IN DOGS

1059

the PVA group (13.6 ± 4.38 and 8.72 ± 4.46 ml/min·100 g wet tissue, respectively). The mean preperfusion values for oxygen consumption in the PA and PVA groups were 3.03 ± 1.05 and 1.87 ± 0.22 ml/min·100 g wet tissue, respectively. In the PA group, oxygen consumption was maintained throughout the experimental period, whereas that in the PVA group tended to increase after perfusion. The stable state of oxygen supply and consumption in the PA group was consistent with the results for dogs with partial portal arterialization reported by Maeda [13].

ATP and its metabolites levels: Approximately 1 g of liver specimen was washed with ice-cold saline to remove blood. One hundred milligrams of liver tissue was then added to 2.5 ml of ice-cold 0.6 M perchloric acid and homogenized with a Potter-Elvehjem homogenizer on ice for 1 min. After neutralization with 0.75 ml of 2 M K2CO3, the homogenate was centrifuged at 3,000 rpm for 10 min to yield a clear supernatant. The supernate was filtered with a 0.45 µm filter and stored at −80°C until assay.

Levels of ATP and its metabolites were determined by the method of Lazzarino et al. [12] with a slight modification. The Gulliver HPLC system (JASCO, Ltd., Tokyo, Japan) with a 4.6 mm × 25 cm silicagel column (Ultraphase ODS Beckman, San Ramon, CA, U.S.A.) kept at 40°C was used. Two mobile solutions consisting of 10 mM tetrabutylammonium hydroxide, 25 mM KH2PO4, and 1.0% methanol (buffer A; pH 6.5); and 5 mM tetrabutylammonium hydroxide, 100 mM KH2PO4, and 30% methanol (buffer B; pH 4.5) were used. The elution with a linear gradient from 0 to 100% of buffer B in buffer A was carried out for 10 to 50 min. A 50 µl sample aliquot was injected and eluted at a flow rate of 1.2 ml/min. The eluent was monitored at 266 nm. The levels of energy charge (EC) were calculated as described by Atkinson [2]: (ATP + 1/2 ADP)/(ATP + ADP + AMP).

Figure 4 shows the changes of ATP and EC levels after perfusion. In the PA group, the ATP level had decreased at 1 hr but recovered to the preperfusion level at 3 hr. On the other hand, the ATP level of the PVA group decreased after perfusion and showed a significantly lower level than that in the PA group. No change of EC level was observed in the PA group, while that in the PVA group decreased to a minimum value at 3 hr after perfusion, showing a significantly lower value than that in the PA group. The maintenance of ATP and EC levels in the PA group were consistent with the results for dogs with partial portal arterialization reported by Maeda [13].

Recovery of the ATP level in the PA group, together with the higher oxygen supply, when compared with the PVA group, suggested that PA provided hepatic cells with sufficient oxygen to maintain the energy production in liver. Since the decrease of EC level to below 0.5 induces irreversible damage to the hepatic mitochondrial function leading to cell death [4, 16, 18], PVA might cause serious damage to the liver of dogs as has been reported in rats [15], probably due to the ischemic condition.

Alanine amino transferase (ALT) activity: The ALT activity was measured as a marker of hepatic damage (Fig. 5). No significant change was observed in the PA group after perfusion. In contrast, the ALT level in the PVA group increased after perfusion as previously reported in dogs and rats [1, 9], with significantly higher values than those of the PA group throughout the experimental period.

Histopathological examination: A small piece of liver specimen was collected from PA and PVA dogs at pre- and 2 hr after perfusion for histological examination. Each liver tissue was fixed in Buan solution and embedded in paraffin.
Five micrometer thick sections were stained with hematoxylin and eosin (Fig. 6). In the PA group, the structure of the hepatic lobule and cord were well preserved with a slight effusion of leukocytes and sinusoidal dilation, while severe sinusoidal dilation and congestion, and granular and vacuolar degeneration were observed at 2 hr after perfusion in the PVA group, indicating serious cell damage.
These results suggested that the liver perfusion system with PA is a good supplementary method to PVA for the maintenance of energy metabolism in liver, and that PA is an available technique for preventing hepatic failure caused by ischemic conditions.

REFERENCES