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Effects of Hepatic Denervation on Ischemia-Reperfusion of the Dogs Liver

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ABSTRACT To investigate the role of the hepatic nerves in ischemia-reperfusion of the liver, we compared hepatic micro-hemodynamics in a denervated liver model to that in the normal liver. The liver was reperfused after total hepatic ischemia for 30 minutes. Hepatic tissue blood flow, index of oxygen saturation, and hemoglobin concentration were measured continuously from 5 minutes before clamping to 120 minutes after reperfusion. In the very early phase following reperfusion (at 1, 2, and 3 minutes), the hepatic tissue blood flow was significantly higher in the denervated group ($P<0.05$). At 2 minutes ($P<0.01$) and at 3, 5, and 15 minutes ($P<0.05$), the index of oxygen saturation in the denervated group was significantly higher. Rapid increaseing blood inflow after reperfusion was observed, possibly due to impairment of intrahepatic microcirculatory regulation caused by denervation. This suggests that the rapid blood inflow during reperfusion after ischemia may modify the reperfusion injury of the transplanted liver grafts.

Key words : 1 Hepatic Denervation
2 Ischemia-Reperfusion Injury
3 Liver Transplantation

INTRODUCTION

Many experiments have been performed to study ischemia-reperfusion of the liver. The hepatic microcirculation is partially regulated by the hepatic nervous system (1, 2). In the transplanted liver grafts, the effects of denervation are difficult to separate from the effects of a temporary interruption of blood flow (3) and surgical damages of the grafts. Then, to investigate the role of the hepatic nervous system in the hemodynamics after ischemia-reperfusion of the liver, we developed a model of the denervated liver using previously reported techniques (3 - 5). There have been several reports describing the hemodynamics and metabolism of the denervated liver (6), but there have been few studies on the hemodynamics after ischemia -reperfusion. Disturbances in the hepatic microcirculation were expected in the denervated liver. Thus, we measured hepatic tissue blood flow (HTBF) (7, 8), index of hepatic tissue oxygen saturation (ISO2), and hemoglobin concentration (IHB) (8, 9) as indicators of hepatic microcirculation.

MATERIALS AND METHODS

Twelve adult mongrel dogs, weighing 10 to

15 kg, were divided randomly into two groups: denervated group (n=6), and control group (n=6). The animals were fasted the night before the experiments. They were anesthetized with intravenous pentobarbital sodium (25 mg/kg body weight). After endotracheal intubation, ventilation was maintained mechanically with room air. A cannula was inserted into the right femoral artery to measure the mean systemic blood pressure (MBP). Saline (0.9%) was infused intravenously during the experiment. All dogs underwent laparotomy (denervation surgery or sham operation).

Experiment

Total hepatic ischemia was induced by clamping the hepatoduodenal ligament at the hepatic hilus. After 30 minutes, the clamp was removed, and the liver reperfused. A laser-Doppler flowmeter probe (Laserflo BRM403A, TSI Co.) was attached to the surface of medial right lobe of the liver to measure hepatic tissue blood flow (HTBF). A spectrophotometer probe (TS-200, Sumitomo Co.) was attached to the surface of medial left lobe to measure index of oxygen saturation (ISO2) and hemoglobin concentration (IHB). Measurements were taken continuously from 5 minutes before clamping to 120 minutes after reperfusion.

Hepatic Denervation

The technique, a combination of surgical neurectomy and phenol application, was chosen because of its completeness (4, 5). After the dogs were anesthetized, the abdomen was opened, and all the hepatic ligaments were severed to free the liver from its attachments to the diaphragm, duodenum, esophagus, and vena cava. The supra- and infrahepatic vena cavae were dissected free. The portal vein and hepatic artery also were dissected free. All structures of the hepatoduodenal ligament except the portal vein and hepatic artery, including the common bile duct, were divided beyond the origin of the gastroduodenal artery. Then, a 90% aqueous phenol solution was applied with a cotton-tipped applicator on and around the portal vein, hepatic artery, and the supra- and infrahepatic vena cavae. After 20 minutes, the experiments were begun (4, 5).

Sham Operation

The time required for denervation was 30 minutes. In the sham group, the same period of time was taken to manipulate the liver and surrounding structures without cutting any of the ligaments. Thus the liver sustained approximately the same degree of mechanical damage in the sham and denervated groups. In addition, saline was applied with a cotton-tipped applicator in the same locations as phenol was applied in the denervated group.

Statistical Analysis

Data were expressed as the mean ± SE. Statistical analysis was performed using the two way analysis of variance (ANOVA) for paired and unpaired data. A P value of <0.05 was considered significant.

RESULTS

Analysis of the Data Before and Just after Denervation

There were no significant differences in MBP, HTBF, ISO2, or IHB before or after denervation. There were no differences between the denervated or sham operated (control) groups (Table 1).

Transitional Changes after Ischemia-reperfusion

During the period of hepatic ischemia, the mean systemic blood pressure fell markedly in both groups. After reperfusion, it gradually recovered, and there were no significant differences between the groups.

On induction of ischemia, the HTBF decreased rapidly, approaching zero. After reperfusion, the HTBF in the denervated group recovered more quickly than that in the controls (Table 2). In the very early phase after reperfusion (at 1, 2, and 3 minutes), the HTBF was significantly higher ($P<0.05$) in the denervated group. After 5 minutes, there were no statistically significant differences.

The index of oxygen saturation followed a similar pattern (Table 2). Immediately after induction of ischemia, the ISO2 decreased to about 10% of the pre-ischemic level. This gradually recovered after reperfusion in both groups. The ISO2 in the denervated group recovered more rapidly. At 2 minutes ($P<0.01$), and 3, 5, and 15 minutes ($P<0.05$), the ISO2 in the denervated group was significant-

Table 1 Comparison of Denervated Livers with Controls

	control group (n=6)	pre-denervation (n=6)	post-denervation (n=6)	p-value
MBP (mm Hg)	115 ± 9.55	135 ± 11.9	107 ± 5.83	0.07
HTBF (ml/min/100g)	13.1 ± 2.56	14.8 ± 4.23	9.80 ± 2.05	0.30
ISO2	21.0 ± 1.87	22.0 ± 2.00	26.0 ± 1.87	0.10
IHB	98.4 ± 3.33	111 ± 7.78	120 ± 5.24	0.24

All data are expressed as the mean ± SE.
No statistical differences were noted between the groups.
P-value: probability of significance of difference between pre- and post-denervation values
MBP: mean systemic blood pressure
HTBF: hepatic tissue blood flow
ISO2: index of oxygen saturation
IHB: index of hemoglobin concentration

Table 2 Comparison of Changes in Hepatic Hemodynamics Following Hepatic Ischemic-Reperfusion in Dogs With Innervated and Denervated Livers

		Reperfusion 1 min	2 min	3 min	5 min	15 min	30 min	60 min	120 min
HTBF	D group	79.7*	108*	112*	99.9	84.6	84.5	77.5	92.7
	C group	28.9	45.7	54.9	82.0	75.5	69.5	55.3	60.2
ISO2	D group	49.6	74.7#	87.8*	94.0*	100*	93.5	100	101
	C group	36.7	44.7	64.5	68.3	75.8	84.7	96.0	101
IHB	D group	79.9	83.2	95.9	96.5	98.6	99.7	101	99.9
	C group	79.4	80.3	87.5	88.8	88.5	92.1	97.9	104

Percentage change from baseline of hepatic tissue blood flow (HTBF), index of oxygen saturation (ISO2), and hemoglobin concentration (IHB) after temporary hepatic ischemia in dogs with (D group) and without (S group) hepatic denervation. In the very early phase following reperfusion (at 1, 2, and 3 minutes), the HTBF were higher in the denervated group ($P<0.05$). At 2 minutes ($P<0.01$) and at 3, 5, and 15 minutes ($P<0.05$), the ISO2 in denervated group was significantly higher.
All data are expressed as the mean and the percentage change from baseline.
*:$P<0.05$, #:$P<0.01$
D group: denervation group
C group: control group
HTBF: hepatic tissue blood flow
ISO2: index of oxygen saturation
IHB: index of hemoglobin concentration

ly higher.

In both groups, the IHB declined to about 70% of the pre-ischemic level after induction of ischemia, and recovered gradually after reperfusion (Table 2). No statistically significant differences existed between the two groups.

DISCUSSION

Previous studies have established that the liver is richly innervated by sympathetic and parasympathetic nerves. The hepatic plexus receives fibers from the celiac plexus, the

vagi, and the right phrenic nerves and forms a thick coat around the hepatic artery. The liver is controlled mainly by the anterior hepatic plexus running from the celiac ganglion along the common hepatic artery, and the posterior hepatic plexus passing to the liver along the portal vessels (10). There are a few nerve fibers in the lesser omentum and the perihepatic ligaments. The physiological function of these nerves has not been established. It must be compared the denervated livers with the innervated livers for the investigation of the roles of the hepatic nerves. A complete denervation of the liver can only be obtained if the liver is autotransplanted or reimplanted after removal. With such procedures, the effects of denervation are difficult to separate from the effects of a temporary interruption of blood flow and mechanical damages of the grafts (3). Several investigators have described techniques for hepatic denervation (3 - 5). We utilized the method of Lautt et al. (4). Complete denervation is reported within 20 minutes after topical phenol application.

We focused on the impairment of intrahepatic microcirculatory control during the early phase of reperfusion. Regional hepatic tissue blood flow measured noninvasively using a laser-Doppler flowmeter has been shown to reflect microcirculation at the level of the sinusoid (7, 8). This technique can be used to measure the total flow volume of capillary blood within a 1 mm circle to a depth of 1 mm. ISO2 measured by a reflectance spectrophotometry reflects oxygen saturation in the hepatic artery, portal vein, and hepatic vein within a defined area of liver (8, 9).

There were no significant differences in MBP, HTBF, ISO2, or IHB between the pre - or post-denervated livers, and the controls. The hepatic hemodynamics in the denervated liver have previously been described (11). No significant change in total liver blood flow was reported. However, the balance of hepatic arterial and portal venous flow did change significantly. Hepatic arterial flow was noted to increase, while portal flow decreased. Stimulation of the hepatic nerves has been shown to decrease liver volume, and result in expulsion of up to half the blood in the liver (12). It may be true that hepatic arterial blood flow increases in the denervated liver, but regional hepatic blood flow did not change.

The duration of hepatic ischemia was set at 30 minutes in this study. Total hepatic ischemia for 30 minutes is critical in the canine model (13). In fact, the mean systemic blood pressure fell by 50%, and bradycardia was observed. None of the animals, however, died during the experiments.

A variation of 15-22% has been reported in the surface perfusion of adjacent sections of the dog liver (14). In fact, it has been concluded that the heterogeneity is sufficiently large so that surface measurements cannot be used as an indicator of total blood flow response (15). Since we also observed heterogeneity in the surface perfusion of the dog liver, vascular flow measurements and oxygen saturation data were derived from comparisons of measurements made at the same point on the liver before and after reperfusion. Although absolute values of HTBF between individual dogs, or in the same dog on different points on the liver cannot be compared, the relative changes in perfusion at a fixed point can be used to estimate the changes in whole-liver perfusion. Furthermore, the same patterns were observed in all dogs in each respective group. In order to observe acute changes in the very early phase of reperfusion, utilization of the fixed probe technique was required. It has been reported that hepatic denervation results in an increase in hepatic blood flow. However, the regional hemodynamics in the denervated liver and the transplanted graft in the early phase of ischemia-reperfusion have not previously been described in detail.

High HTBF and ISO2 imply a greater blood volume at the level of the hepatic sinusoid. There is now considerable evidence that hepatic parenchymal cells, Kupffer cells, and endothelial cells receive efferent innervation of predominantly sympathetic or parasympathetic origin depending on the species (11). Previous studies have shown neither dorsal root fibers nor sympathetic vasodilator nerve fibers in splanchnic vessels (16). Thus, it can be postulated that the observed increase in HTBF and ISO2 is due

to diminished vasoconstrictor tone at the level of the sinusoid. Since there were no differences in HTBF and ISO2 30 minutes after reperfusion, it is proposed that the initial response is under neural control, whereas the later effects are the result of humoral factors.

After reperfusion, the HTBF and ISO2 in the denervated group recovered much more rapidly than in the control group, that is, rapid increase of the blood inflow in the early phase of reperfusion is occured in the denervated liver. It is considered that there are some demerits for the denervated liver after ischemia-reperfusion. It is a possibility that oxygen free radicals were produced in large quantities in the denervated liver by the rapid and high influx of highly oxygenated blood. The second possibility is that the more intense liver injury was induced by a greater influx of harmful substances derived from the congested intestine. The third possibility is that sinusoidal congestion after reperfusion was greater in the denervated liver. Because the perihepatic lymphatic vessels were divided during the surgical neurectomy, hepatic outflow was probably lower. Since the lymphatic outflow of the denervated liver was disturbed, a greater degree of sinusoidal congestion may have occurred, resulting in greater liver injury.

In conclusion, rapid increase of blood inflow was observed in the denervated liver during reperfusion, probably as a result of impairment of intrahepatic microcirculatory regulation. Furthermore, as it has been speculated that ischemia-reperfusion is one of the most important causes of primary graft non-function in hepatic transplantation, we hypothesize that the lack of a neural response, that regulates rapid increase of blood inflow to the graft, may contribute to primary graft failure.

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