

Portal Hyperfusion or Hepatic Venous Congestion: Which One Affects Kupffer Cell Function More?

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Abstract

Objectives: Because of their effects on the liver parenchyma after surgery, portal hyperperfusion and hepatic venous congestion are challenging problems for hepatobiliary surgeons. However, the effects of those conditions on Kupffer cells have not been established. The aim of this study was to investigate the effects of vascular streams modified by portal hyperperfusion and hepatic venous congestion on Kupffer cell function.

Materials and Methods: Thirty rats were allocated into 3 groups of 10 rats each and were subjected to right portal vein ligation to induce hyperperfusion in the left lobe of the liver (group 1), occlusion of the right hepatic vein to produce venous congestion (group 2), or sham operation (controls; group 3). After 72 hours, the right and left liver lobes of the subjects were submitted separately for scintigraphic and histopathologic evaluation, and the radiocolloid uptake per gram of liver tissue and the number of Kupffer cells per square millimeter were calculated.

Results: The mean technetium-99m labeled sulfur colloid uptake values of the liver tissue per gram were 0.126 ± 0.038 for group 1, 0.106 ± 0.032 for group 2, and 0.110 ± 0.031 for group 3. Portal hyperperfusion significantly increased the technetium-99m labeled sulfur colloid uptake of the liver tissue per gram ($P = .043$). The mean number of Kupffer cells per square millimeter was calculated

for each group as follows: $321 \pm 094 \times 10^{-6}$ for group 1, $369 \pm 083 \times 10^{-6}$ for group 2, and $355 \pm 096 \times 10^{-6}$ for group 3. Both vascular streams produced no significant effects on the number of Kupffer cells ($P > .05$).

Conclusions: In this experimental model, portal hyperperfusion affected Kupffer cell function more than did hepatic venous congestion.

Key words: Kupffer, Portal hyperperfusion, Hepatic venous congestion

Portal hyperperfusion and hepatic venous congestion are challenging problems for hepatobiliary surgeons. Because the cumulative knowledge about hepatic surgery has increased as has the performance of extended resection and transplant procedures, the importance of inflow and outflow modalities has been realized. Because of their direct impact on functional capacity, those alterations are virtually as effective as remnant or transplanted liver volumes. Nevertheless, the outcomes of such undesired conditions are difficult to predict during the preoperative period, and studies on that topic have been focused primarily on the deterioration of parenchymal cells. The effects of portal hyperperfusion and hepatic venous congestion on the function of nonparenchymal cells (Kupffer cells in particular) remain poorly defined (1-3).

Kupffer cells, which are resident liver macrophages, have a unique role in immunomodulation, and the mediators released from those cells disrupt the functional integrity of hepatocytes (4-7). Therefore, clarifying the functional diversification of Kupffer cells under modified vascular streams may clarify the characteristics of those cells after extended resection or a transplanted small graft. Our study investigated the effects of portal hyperperfusion and hepatic venous congestion on Kupffer cell function.

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Materials and Methods

Animals and preparation

Thirty adult male Wistar rats (weight range, 200 to 250 g) were used in this study. The animals were obtained from the Ege University Department of Experimental Surgery, Animal Breeding, and Research Laboratory. The experimental procedures of the study were approved by the Institutional Animal Ethics Committee and were performed according to the local guidelines for the use and care of laboratory animals. All rats were kept in single cages at room temperature and were exposed to a 12-hour alternating light-and-dark cycle. The rats were fed standard rat chow and water ad libitum. Twelve hours before the experiment, they were fasted of food but had free access to water.

Experimental design

The subjects were randomly allocated into 3 groups (10 rats in each group) and were subjected, under sterile conditions, to right portal vein ligation to induce hyperperfusion in the left lobe of the liver (group 1), occlusion of the right hepatic vein to produce venous congestion (group 2), or sham operation (controls; group 3). A midline laparotomy was performed after each subject had been anesthetized with intramuscular ketamine 10 mg/kg. In group 1, the right portal vein of each rat was ligated with 5.0 silk, and in group 2, the right hepatic vein was occluded with an 11-mm Auto-Suture hemostatic clip (Auto-Suture, Norwalk, Connecticut, USA). The rats in group 3 underwent only laparotomy. The abdominal incisions were closed in 2 layers with continuous 3.0 silk sutures.

After 72 hours, syringes containing 0.3 mCi (11.1 Bq) technetium-99m labeled sulfur colloid (Tc-99 SC) were prepared for each rat before scintigraphic evaluation. The quantities of activity in the syringes before and after injection were measured by a δ camera (Toshiba, GCA – 602A, Japan) with a static imaging protocol of 0.6 minutes (128 × 128 matrix; low-energy all purpose collimator). The rats were treated with ketamine to induce anesthesia, and 5 minutes after an intracardiac injection of Tc-99 SC, they were killed by cervical dislocation. Total hepatectomy was performed in all groups, and the livers from groups 1 and 2 were divided into right and left lobes. The left lobes of the rats in group 1, the right lobes in group 2, and the whole liver in group 3 were subjected to the same static imaging protocol

of 0.6 minutes. The Tc-99 SC uptake of the liver tissue samples was calculated by the following formula:

The Tc-99 SC uptake of the liver specimen equals the count rate of the liver specimen divided by the difference between the count rates of full and empty syringes.

The liver specimens were weighed on a precision balance, and the Tc-99 SC uptake of each specimen was divided by those values to calculate the activity of the liver tissue per gram to ensure standardization among the groups.

Histopathology

The liver samples were fixed in formaldehyde, processed routinely, and embedded in paraffin. Sections 3 μ m thick were cut and stained with hematoxylin-eosin, reticulum, and periodic acid Schiff's reagent after diastase reaction (Figure). The stained sections were examined under a light microscope (Olympus, BH-2, Japan), and Kupffer cells were evaluated on a monitor (JVC, TM210-PS, Japan) with a camera (JVC, TK890-P, Japan) that was connected to the microscope. The Kupffer cells were counted in 20 randomly selected high-power field areas in each tissue section with 1 section per animal (40 × objectives), and the number of Kupffer cells per square millimeter was calculated.

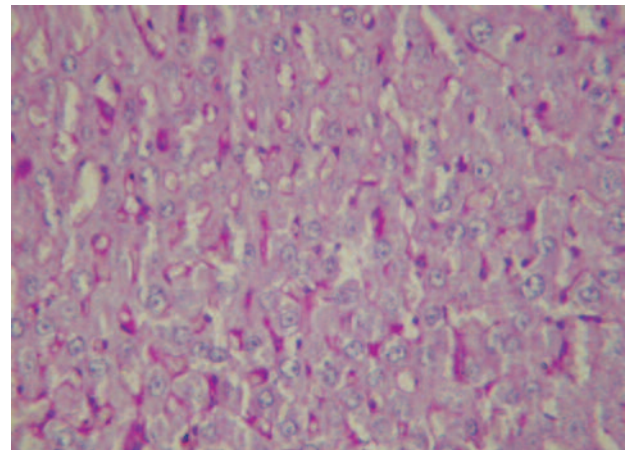


Figure. Pink-red staining of Kupffer cells with periodic acid Schiff after diastase reaction (d-PAS, x20).

Statistical analyses

All numerical data were expressed as the mean $\pm$ standard deviation. Statistical differences between groups were assessed with the Mann-Whitney U test, and differences were considered statistically significant at a *P* value of < .05.

Results

The mean values of the groups after scintigraphic and histopathologic assessments are summarized in the Table. The mean Tc-99 SC uptake of the liver tissues per gram in each group was 0.126 ± 0.038 for group 1, 0.106 ± 0.032 for group 2, and 0.110 ± 0.031 for group 3. The mean rank was 12.38 for group 1 versus 7.20 of controls and 9.65 for group 2 versus 11.35 of controls. The differences were significant for group 1 (Mann-Whitney *U* test = 17.00, $P = .043$) but not for group 2 (Mann-Whitney *U* test = 41.50, $P = .529$). Those results revealed that portal hyperperfusion significantly increased the Tc-99 SC uptake of the liver tissues per gram, but hepatic venous congestion did not do so.

Table. Mean values in the study groups after scintigraphic and histopathologic assessment

Variables	Portal hyperperfusion	Hepatic venous congestion	Control group
Mean Tc-99 SC uptake	$0.126 \pm 0.038^*$	0.106 ± 0.032	0.110 ± 0.031
Mean KC no./mm ²	$(321 \pm 094) \times 10^{-6}$	$(369 \pm 083) \times 10^{-6}$	$(355 \pm 096) \times 10^{-6}$

Abbreviations: Tc-99 SC: Technetium-99m labeled sulfur colloid KC: Kupffer Cells $P < .05^*$

The mean number of Kupffer cells per square millimeter was calculated for each group as follows: $321 \pm 094 \times 10^{-6}$ for group 1, $369 \pm 083 \times 10^{-6}$ for group 2, and $355 \pm 096 \times 10^{-6}$ for group 3. The mean rank was 6.81 for group 1 versus 11.65 of controls and 11.40 for group 2 versus 9.60 of controls. Both portal hyperperfusion and hepatic venous congestion did not produce a significant effect on the number of Kupffer cells (Mann-Whitney *U* test = 18.50 for group 1 and 41.0 for group 2, $P > .05$ for both). However, there was a remarkable decrease in the Kupffer cell population as a result of portal hyperperfusion in the control group ($P = .055$).

Discussion

The major finding of this study was that portal hyperperfusion produced a greater impact on Kupffer cell function than did hepatic venous congestion. Under excessive portal venous inflow, Kupffer cell function was stimulated, however; the number of those cells tended to decrease. Liver failure after resection or transplant is often caused by technical difficulties involving volume, portal inflow,

or hepatic venous outflow (8-12). Remnant or transplant liver volume can be detected by volumetric measurements with high accuracy during the preoperative period (13,14). With regard to portal hyperperfusion and hepatic venous congestion, it is difficult to anticipate harmful effects, such as those caused by increased sinusoidal pressure (which in turn produces a delay in or failure of synthetic ability and increases the effects of cytolytic enzymes and cholestasis on hepatocyte function) (10,15,16). Surgical techniques such splenic artery ligation (17) or embolization (12), splenectomy (18), portocaval (19) or mesocaval (20) shunting for portal overperfusion, reconstruction of middle hepatic vein and its tributaries (21), or the use of interpositional grafts (22) to relieve hepatic venous congestion have been suggested to overcome those issues.

The activity of Kupffer cells (which account for the greatest population of nonparenchymal cells), like that of hepatocytes, is extremely important. Activated Kupffer cells are major sources of potent inflammatory mediators such as reactive oxygen species, cytokines (especially interleukin-6 and tumor necrosis factor- α), eicosanoids, and chemokines all playing miscellaneous roles in various types of liver injury (5, 6, 23, 24). Recent evidence that reveals the role of activated Kupffer cells in apoptosis, T-cell response, rejection, and ischemia-reperfusion injury emphasizes the importance of these cells in liver transplant (25-28). However, the effects of portal hyperperfusion and hepatic venous congestion on Kupffer cell function after major hepatic surgery have not been determined.

Hepatobiliary scintigraphy is a noninvasive objective method used to assess the liver in vivo in clinical (29,30) and experimental (31) studies. Tc-99 SC, which is frequently used to evaluate hepatic reticuloendothelial system function, differs from other radiocolloids. The clearance mechanism for that agent does not require active phagocytosis or pinocytosis. Therefore, the uptake of that tracer accurately reflects hepatic blood flow (32). For those reasons, Tc-99 SC scintigraphy was the method chosen for the evaluation of Kupffer cell function in our study.

This experimental study was designed to assess the isolated effects of modified vascular streams on Kupffer cell function with the aim of excluding the effects of liver volume. Therefore, a resection model

was not used. Because our study is not a transplant model, potential immunologic mechanisms such as rejection or ischemia-reperfusion injury, both of which can activate Kupffer cell function (23,25) were excluded. On the other hand, excluding parenchymal resection counterworks with regard to measuring the pure effects of venous inflow and outflow modifications. Both right and left liver lobes have separate portal branches and hepatic veins. However, in the whole liver, interlobar venous connections decrease elevated sinusoidal pressure caused by portal hyperperfusion or hepatic venous congestion. As might be expected, the detrimental effects of portal hyperperfusion occur before the buffer effect of that network. Nevertheless, increased sinusoidal pressure produced by outflow obstruction leads to regurgitation (33), and those connections quickly alleviate that congestion via the lower gradient toward the counter lobe. Those connections are considered the determinants of this model by means of the natural position between the 2 vascular structures. In partial livers in resection models, the ligation of those connections eliminates the buffer effect and exacerbates the influences of inflow and outflow problems. However, volume may affect the results in different ways. Within this context, the design of the experimental model will be utmost causative.

In our study, portal hyperperfusion significantly induced / increased the activity of Kupffer cells. That finding supports the results of studies emphasizing the undesirable consequences of excessive portal inflow (1, 2, 11, 17). This increased activity of nonparenchymal cells should be assessed in addition to parenchymal injury. Ischemia-reperfusion studies indicate that microcirculatory changes on cellular levels can reach maximum levels even after 48 hours of reperfusion (34). Although our study was not an ischemia-reperfusion model, we considered the data from those studies and killed our subjects after 72 hours. Though it was not statistically significant, the number of Kupffer cells in the portal hyperperfusion group decreased in addition with the increased uptake of those cells. That decrease was attributed primarily to depletion after overstimulation. The ability of Kupffer cells to migrate along sinusoidal walls has been suggested (35). Therefore, the decrease in Kupffer cell number may be a result of the removal of those cells from sinusoidal spaces during downregulation.

The limitations of our study include the lack of measurement of the mediators released from Kupffer cells. Actually, that preference reflects the main goal of our research. As mentioned above, the aim of our study was to investigate the effects of modified vascular streams on the population of a specific cell type rather than the secondary effects of those cells by means of various mediators.

In summary, it should not be forgotten that liver failure after surgery is a complex event and that the influences of modified vascular streams inevitably interfere with the effects of remnant or transplanted liver volume in clinical practice. After we had evaluated the effects of inflow and outflow patterns in the experimental model of this study, we concluded that portal hyperperfusion affected Kupffer cell function more than did hepatic venous congestion.

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