

Original Article

Rat liver sinusoidal dilatation induced by perfusion in vitro through portal vein alone, hepatic artery alone, and portal vein together with hepatic artery

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Abstract: The techniques for liver perfusion have been recommended in order to protect liver grafts prior to transplantation. We compared portal vein alone, hepatic artery alone, and dual perfusion through portal vein together with hepatic artery perfusion techniques in rat livers analyzed in histology. Rat livers were treated for 30 min in situ warm ischemia and excised and directly placed to mimic conventional cold storage for 4 hours followed by different routes of cold perfusion. In three experimental groups, five livers were given portal vein perfusion in group one. In group two, five livers were subjected to hepatic artery perfusion and in group three five livers received both portal vein and hepatic artery perfusion. Histological samples were taken before the beginning of the perfusion, 30 minutes, and 60 minutes after starting of the perfusion. Rat livers, received portal vein perfusion in group one, revealed remarkable sinusoidal dilatation increasingly with augmented perfusion time. The livers through both the portal vein and the hepatic artery showed significantly reduced sinusoidal dilatation compared to the portal vein perfusion in group one. Moreover, the liver perfusion through the hepatic artery demonstrated the lowest sinusoidal dilatation when compared to the portal vein perfusion and both the portal vein and the hepatic artery perfusion. This is the first evidence that the hepatic artery perfusion alone is the best of three perfusions by showing in sinusoidal dilatation. Also, we clearly demonstrate that the severity of the sinusoidal dilatation is tightly related to the time of portal vein perfusion.

Keywords: Hypothermic perfusion, hepatic endothelial cells, sinusoid dilatation, liver histology

Introduction

There are several solutions for 'ex vivo preservation' prior to liver implantation in order to improve or rescue injured liver grafts. However, there are only two or three routes to conduct the liver perfusion through the portal vein infusion [1-4] or a combination of the portal vein and the hepatic artery [5-8]. The benefits of the preservation have been shown in various animal models, for the human liver transplantation these techniques have been shown the restrictions [7, 8].

Researchers and clinicians have developed a variety of methods to face the growing demand of the liver transplant. Many strategies have been involved, such as using different tempera-

tures or different solutions or with/without oxygenated solution in the perfusion [2, 4, 6, 7, 9-11] etc. Unfortunately, the conclusive answer for these approaches is still unclear.

Sinusoidal dilatation (SD) is basically characterized by enlarged hepatic sinusoids mainly shown by a predominant histopathological feature [12-14] and is thought as artificial or non-specific changes [12]. But more and more evidence has been found that SD is associated with inflammatory diseases [12, 15-20], portal hypertension [16, 20, 21] and vascular disorder [20, 22]. SD has been found in several liver perfusion studies without mentioning [23-25].

In this short article, we report that liver perfusion is tightly related to SD confirmed with his-

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tological analysis in our rat model. We also investigated the relationship of SD and different routes of perfusions with portal vein alone, hepatic artery alone, and both of portal vein and hepatic artery at different time points compared in histology respectively.

Materials and methods

Animals

Male Sprague Dawley rats (280-330 g) were used in all experiments. Animals were maintained in the standard laboratory diet and water according to the Anhui Medical University. All animals used in all experiments were approved by the animal ethics committee in Anhui Medical University. Anaesthesia was done with pentobarbital sodium (50 mg/kg intraperitoneal injection). Heparinization was done after anesthesia intravenously.

Study design

A part of the established rodent donation after cardiac death (DCD) model [22, 23] has been adopted in our experiments with prior heparinization. The aortic and portal vein blood pressure was measured before the time of cardiac arrest. The period of *in situ* warm ischemia began from the time of cardiac arrest.

Hypothermic perfusion (HP) was performed with pre-cooling phosphate buffered saline (with ice cube floating in a container and the temperature was kept from 1 to 4°C). All DCD livers were divided into three groups. (1) portal vein alone (PV, n=5), (2) a combination of portal vein and hepatic artery (PV+HA, n=5), and (3) hepatic artery alone (HA, n=5). All the livers were exposed to 30 minutes of *in situ* warm ischemia followed by 4 hours cold storage and then cannulated and perfused [26].

Hypothermic perfusion

Pre-cooling phosphate buffered saline was used for HP with heparin (1000 U/l). Standard bench preparation was performed and then the blood pressure in the aorta and the portal vein was measured. Cannulation of the portal vein and hepatic artery were accomplished with perfusion cannulas of appropriate size. All bench work and cannulation of the liver was followed with the criteria of surgery [27]. The portal vein and the hepatic artery underwent continuous

HP with nearly physiological conditions in pressure and volume. HP was performed for 1 hour.

Arterial and portal vein pressure measurement

The abdominal aorta was exposed by a midline incision. The mean liver artery pressure was presented by the aorta arterial pressure measured by a 21-gauge needle connected with a polyethylene catheter (1 mm in diameter) vertically placed with a ruler and filled with PBS (phosphate buffered saline) containing heparin (1 unit/ml) for each rat in the experiments. The needle was inserted into aorta artery proximately 5 mm over the right renal artery branch. The pressure of portal vein was done in a similar way to the aorta arterial pressure for each experimental rat.

Liver tissue sampling

Samples of liver tissue were all taken from the edge of median lobe at pointed time for histological analysis respectively. The samples of each group were collected from the location at the same pointed time.

Histological analyses

Liver tissues were fixed in 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline), embedded in paraffin, sectioned in 4 μ m, and stained with hematoxylin and eosin. Sinusoidal dilatation liked areas between the hepatocyte cords in each slide was calculated by ImageJ software [28-31]. The degree of sinusoidal dilatation liked areas was presented as percentage of the areas to the whole tissue area measured.

Statistical analysis

Statistical evaluation was done using the unpaired, two-tailed, Student's *t*-test, and data presented are means \pm SE. *p* value is shown on columns analyzed.

Results

Rat aorta and portal vein pressure

In order to ensure that the perfusion of rat liver is near physiological conditions, the pressures of abdominal aorta artery that represents the hepatic artery pressure and portal vein for each experimental rat were firstly measured before

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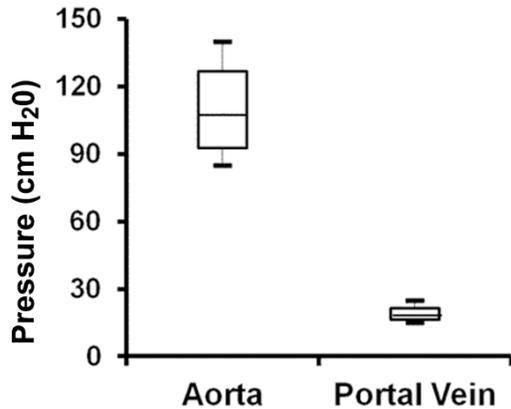


Figure 1. Rat aorta and portal venous pressure. The aortic and portal vein blood pressure of all rats (n=15) were measured before experiment. A 21-gauge needle connected with polycarbonate tube (1 mm in diameter) and was inserted into aorta artery or portal vein. All data in the figure are mean \pm SEM.

the cardiac arrest. As arterial blood is pulsating, arterial blood pressure is composed of a baseline. The baseline of arterial blood pressure was 80 mmHg (108 ± 18 cm H₂O, n=15) and the portal vein pressure was 13 mmHg (18 ± 3.9 cm H₂O, n=15) shown in **Figure 1**. The results of our blood measurement were lower than the other studies [32, 33] and it might be due to our polyethylene catheter with higher resistance to the solution filled in the tube.

Portal vein alone perfusion induced hepatic sinusoidal dilatation liked alteration

As a first step toward imitating the conventional cold storage and perfusion, portal vein was connected to a cold perfusion tube with the pressure of 11 mmHg (15 cm H₂O) controlled by gravity at 10 ml/min for 60 minutes after 30 minutes warm ischemia and 4 hours cold storage. The liver tissues histologically analyzed showed that the portal vein perfusion induced pronounced sinusoidal dilatation liked alteration, such as 60 minutes after perfusion induced 36% sinusoidal dilatation liked alteration, while approximately 2% was found before the perfusion (**Figure 2A** and **2B**).

Considering the alteration after 60 minutes in the portal vein perfusion, we assumed the sinusoidal dilatation liked alteration must derive from the perfusion. Therefore, we repeated the

experiments (n=5) and took the liver tissues (order seen in method and material section) after 30 minutes of the perfusion and analyzed in a similar way. The results have shown that 28% of the sinusoidal dilatation liked area was found whereas 36% was exhibited in the 60 minutes. With regarding such differences of the percentages of the sinusoidal dilatation liked areas among the control, 30 minutes, and 60 minutes, the portal vein alone perfusion induced the alteration and the severity of the sinusoidal dilatation liked alteration was paralleled with the increased time (shown in **Figure 2A** and **2B**). The significance of values was indicated on the tops of columns. These results directly indicated that portal vein perfusion with even the pressure lower than physiological pressure still harmed the liver tissues shown by increased sinusoidal dilatation liked alteration.

Additionally, we also observed histological features in the portal vein perfused samples, such as lose of a well-preserved lobular/parenchymal architecture in the portal vein perfusion (**Figure 2**) comparing taken before the perfusion and even found a lot of sinusoidal endothelial cells peeling off. Representative histological alteration of the sinusoidal endothelial cell peeling off is shown (**Figure 2C**).

Dual perfusion through portal vein together with hepatic artery induced less sinusoidal dilatation liked alteration

The artery facing much higher blood pressure in general is much stronger than the vein physically and anatomically. Therefore, we assumed whether it could be possible or not that we let the hepatic artery take a certain volume of perfusion solution to reduce the stress from the portal vein. We performed dual perfusion through portal vein together with hepatic artery (via aorta) with dividing 5 ml/min with 80 cm H₂O (58 mmHg) and the portal vein was 5 ml/min with 15 cm H₂O (11 mmHg) [34]. The pointed time of 60 minutes of the perfusion was chosen for histological comparison. The statistically analyzed results had shown that the hepatic artery perfusion did not increase the sinusoidal dilatation liked alteration. Actually, this arterial perfusion even decreased the induction of sinusoidal dilatation liked alteration significantly while compared with the one done by the portal vein alone (**Figure 3**).

Portal vein perfusion induced sinusoidal dilatation

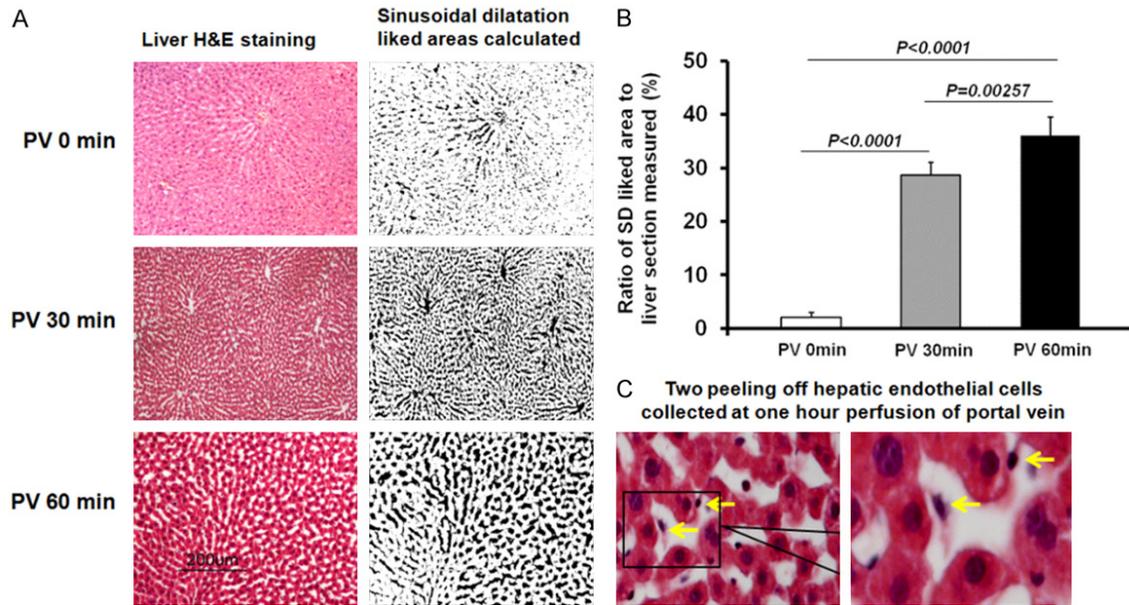


Figure 2. Portal vein perfusion induced hepatic sinusoidal dilatation. The DCD livers were perfused by portal vein for 60 min after 30 min warm ischemia and 4 h cold storage. The liver tissue samples were taken at 0 min, 30 min and 60 min. (A) Hematoxylin-eosin staining (left) and the areas of sinusoidal spaces (right) analyzed by ImageJ in liver tissues; scale bars in (A), 200 μ m in the length ($\times 200$). (B) Quantification of sinusoidal dilatation liked areas was described as the ratio of sinusoidal spaces to entire liver section measured. (C) Endothelial cells in sinusoidal spaces. Two peeling off hepatic endothelial cells collected at one hour perfusion of port vein. Statistical difference was shown on the columns analyzed. All data in (B) are mean \pm SEM.

Hepatic artery perfusion alone reduced sinusoidal dilatation liked alteration

Since our dual perfusion reduced the sinusoidal dilatation liked alteration, we wanted to see how the perfusion only via the hepatic artery. We repeated the experiments of rat liver perfusion only via the hepatic artery after warm ischemia and cold storage. We found that the hepatic artery, as the accessing route to perfusion liver, reduced sinusoidal dilatation liked alteration maximally (**Figure 3**). The ratio of SD liked areas to rat liver section measured was only 26% in the hepatic artery perfusion while 36% and 32% in the portal vein alone and the dual perfusion respectively. The remarkably reduced liver tissue injury was carried out from the hepatic artery perfusion compared with the dual perfusion and the portal vein perfusion.

Discussion

We looked for a conclusive comparison among different accessing routes of hypothermia perfusion in this series of experiments. This is the first investigation of liver perfusion through hepatic artery alone in rat model. We found that it is possible to perfuse the rat liver at a

low temperature of 1.5 to 4°C with pressure lower than physiological pressure through hepatic artery. The gravity controlled pressure was straightforward at the desired level. The reason why we chose 60 minutes for perfusion time was that the histological features of the sinusoids which appeared wider have been shown clearly at this time point in the samples after warm ischemia and cold storage.

Our data clearly indicated that the portal vein perfusion injured the liver sinusoids and the severity of the sinusoids was paralleled to the increased perfusion time. These meant that even the pressure lower than that of physiology, the inflow might still be a harmful factor to the liver endothelial cells at the status we created for. The observation of hepatic endothelial cells peeling off from the portal vein perfusion was direct evidence verifying that the consistent inflow pressure was doing the opposite job to our wish. The data from the reduced duration of the portal vein perfusion and from the reduced volume in the dual perfusion showed the reduced liver sinusoid injury. Our results clearly pointed out that the liver hepatic endothelial cells were very fragile after 30 minutes

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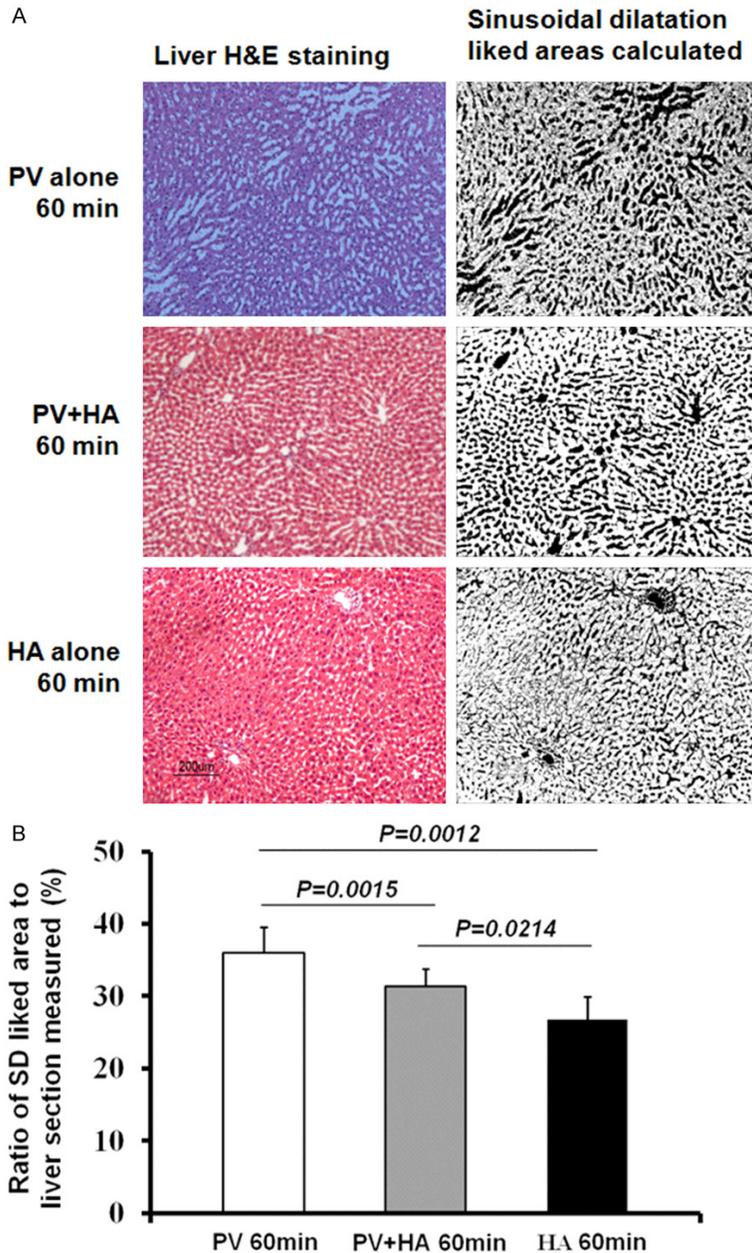


Figure 3. Hepatic artery perfusion protected liver against perfusion injury. The DCD livers were perfused by portal vein alone (group 1), both hepatic artery and portal vein (group 2) or hepatic artery alone (group 3) for 60 min after 30 min warm ischemia and 4 h cold storage. The liver tissue samples were taken at 60 min after perfusion. (A) Hematoxylin-eosin staining (left) and the areas of sinusoidal spaces (right) analyzed by ImageJ in liver tissues of three groups; scale bars in (A), 200 μ m in the length ($\times 200$). (B) Quantification of sinusoidal dilatation liked areas was described as the ratio of sinusoidal spaces to entire liver section. Statistical difference between groups was shown on the columns analyzed. All data in (B) are mean \pm SEM.

of warm ischemia and 4 hours of cold storage in our rat model which is also considered by others [34].

junctional communication [43]. Therefore, our data showed the widen sinusoids induced by portal vein perfusion was harmful to the hepatic

Sinusoid dilatation has been described as sinusoidal spaces more than one hepatocyte plate in width without any other histological abnormality [35]. It was very hard to determine which sinusoid could be calculated as sinusoidal dilatation and which one was not. Thanks for Image J software [28-31] which allowed us to calculate the spaces between hepatocyte cords and gave us reliable and consistent results. A part of the results were tested and compared with our manual analyses resulted in high similarity (data not shown). The sinusoidal dilatation has been described as post sinusoidal outflow block shown by enlarged sinusoids determined by histopathological features [35], while a study has shown that the sinusoidal dilatation is not involved in venous obstruction [36]. It should be pointed out that the sinusoidal dilatation could be induced by artificial tearing during the process for histological study [14], we checked our control samples (collected before the perfusion) and did not find even one with so called sinusoidal dilatation liked area. Considering the sinusoidal dilatation are gradually recognized as abnormalities related to diseases [15, 16, 35, 37-39] or to liver sinusoid endothelial cell injury from reperfusion [27, 40] and reperfusion injury from others [41, 42]. The widen sinusoids would interfere the gap junction of the hepatic endothelial cells forming the sinusoids and consequently affect the gap

endothelial cells after warm ischemia and cold storage in our rat model.

The hepatic artery alone perfusion reduced the endothelial injury was remarkably shown with the decreased sinusoidal dilatation liked area in this study, whereas the portal vein perfusion as well as dual perfusion [6, 8-10] have been often used in order to protect hepatocytes from perfusion injury [1, 3, 44]. The differences of an artery and a vein are various from the view of physiology and anatomy and the arteries are much stronger and more durable than the veins. Our data suggest that the hepatic artery alone perfusion should be considered firstly, but it is rarely used in the field [45].

It is extremely important to evaluate liver graft quality after hypothermic perfusion in order to avoid associations related with primary non-function, early allograft dysfunction, and biliary complications after liver transplantation [46]. We believe that the altered sinusoids/sinusoidal dilatations derived from the cold perfusion would change the microcirculation consequently affecting the complex function of the liver in biosynthesis, metabolism, clearance etc [47, 48], while the hepatic endothelial cells that form the hepatic sinusoids are positioned in a single layer.

It is obviously that the single layered hepatic endothelia cells after warm ischemia and cold storage could be very delicate and easily broken. The hepatic endothelial cells play an important role in frontier to protect the hepatocytes [49]. Therefore, the sinusoid status in histology should be a parameter in the assessment of liver quality together with other biomarkers [50].

In conclusion, this study showed that the hepatic endothelial cells were very fragile and the sinusoids were widen from the hypothermic perfusion and the hepatic artery alone perfusion presented minimal sinusoidal endothelial cell injury. Therefore, the route and duration of perfusion could be important to rescue liver organ in addition to many other factors. Further evaluations of our results in our whole-organ-system culture *in vitro* are expected.

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Disclosure of conflict of interest

None.

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