Effect of endotoxin on portal hemodynamic in rats

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Abstract

AIM: To study the effects of endotoxin on portal hemodynamic of normal and noncirrhotic portal hypertensive rats.

METHODS: Normal rats were intraperitoneally injected with 0.1, 0.25, 0.5, 1.0, 2.0, 4.0mg·kg⁻¹ of lipopolysaccharide (LPS) respectively, portal vein ligation (PVL) and intrahepatic portal occlusion (IPO) rats as well as sham-operated rats were treated with an intraperitoneal injection of 1.0mg·kg⁻¹ of LPS, the portal vein pressure (PVP), portal venous flow (PVF), inferior vena cava pressure (IVCP) and portal vein resistance (PVR) were detected 4 hours after injection.

RESULTS: PVF of the 5 groups of rats accepting intraperitoneal injection of LPS were increased from 14.0 to 18.0, 22.2, 26.2, 34.8, 39.6, 38.8mL·min⁻¹ 4 hours after injection of LPS (P<0.01). PVP of the 4 groups of rats accepting more than 0.1mg/kg·b.w of LPS was increased from 1.04 to 1.25, 1.50, 1.80, 1.95, 2.05 kPa (P<0.01). The increments of PVF and PVP were in a dose-dependent manner of LPS. PVR of the 5 groups of rats was decreased from 51 to 42.44, 48.45, 44.47kPa·min⁻¹·L⁻¹ (P<0.05) and no dose-dependent manner was observed. PVF of PVL, IPO and sham-operated rats increased from 22.6 to 32.8, 22.0 to 28.0, 14.0 to 34.8mL·min⁻¹ (P<0.01), and PVP increased from 1.86 to 2.24, 1.74 to 1.95, 1.04 to 1.80kPa (P<0.01), PVR decreased from 71 to 61, 67 to 61, 52 to 44kPa·min⁻¹·L⁻¹ after intraperitoneal injection of 1mg·kg⁻¹ of LPS. The increments of PVF and PVP of PVF and IPO rats were significantly less than the sham-operated rats (P<0.01). No significant difference between the amounts of PVR decreased in the two groups of PHT model rats and sham-operated rats (P>0.05) after intraperitoneal injection 1mg·kg⁻¹ of LPS.

CONCLUSION: Endotoxin could prompt portal hypertension of the normal and noncirrhotic portal hypertensive rats by increasing portal blood flow mainly.

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INTRODUCTION

Endotoxin is lipopolysaccharide (LPS), a component of the outer membrane of the Gram-negative bacteria, which is released from the Gram-negative bacterial cell wall. Its functional component is lipoid A. Many researchers have discovered that endotoxemia can lead to an alteration of systemic hemodynamics and some organs’ blood circulation such as the lungs, liver and kidney[1-4]. However, some researchers have displayed evidence against a role for endotoxin in the hyperdynamic circulation of rats with prehepatic portal hypertension[5]. The activation of endotoxin occurs through a series of vaso modulators such as nitric oxide (NO), endothelin and others[6-11]. These vaso modulators could modulate portal venous flow (PVF), portal vein resistance (PVR) and/or portal vein pressure (PVP). In patients suffering from liver cirrhosis with PHT, endotoxemia is often present and might contribute to the development of liver cirrhosis and PHT[12-14]. Whether or not PHT models without liver cirrhosis are more sensitive to endotoxin is still unclear[15,16]. Little has been done to study the effects of various dosages of LPS on portal hemodynamics. So, to detect what role endotoxin plays in PHT, we designed the following experiments to discover the effects of various dosages of LPS on the portal hemodynamics of both normal rats as well as non-cirrhosis PHT rats.

MATERIALS AND METHODS

Animals

Female Sprague Dawley rats weighing 200-250g were obtained from the Laboratory Animal Center of Sun Yat-sen University, and fed with standard rat chow. (1)Surgery was performed as in Yachida’s method[17]. Under penbarbital (50mg·kg⁻¹, intraperitoneal injection) anesthesia, the portal vein was isolated and a single ligature placed around both the portal vein and a 16-gauge needle. The needle was ligated together with the portal vein and immediately removed to allow the portal vein to expand to the limit imposed by the ligature. A catheter was inserted through the mesentery vein into the portal vein and another into the inferior vena cava. Pressure transducers (Philips CM 130) recorded PVP and IVCP. PVF was recorded with an electromagnetic flow meter (Nihonkoden). The abdomen was closed and the rats were allowed to recover for 2 wks. Sham-operated rats, surgery consisted of dissection and visual inspection of the portal vein without ligature. (2)Surgery was performed as in Li’s et al[18] method. Under penbarbital anesthesia as above, microspheres (about 2×10⁴ each time) of Sephadex LH-20 (Pharmacia) were injected into the mesentery vein; injection was repeated 5 times. The portal venous and vena cava pressure were recorded as above. The abdomen was closed and the rats were allowed to recover for 2wks. Sham-operated rats above were used as a control.

Effects of LPS on portal hemodynamics

Normal rats were divided into seven groups, each group containing five rats. Rats were intraperitoneally injected with LPS(from Escherichia coli serotype, Sigma) at dosages of 0.1, 0.25, 0.5, 1.0, 2.0, 4.0mg·kg⁻¹ respectively. Equivalent volumes of saline were intraperitoneally injected as a control. 4h later, anesthesia and operation were manipulated as above. A catheter was inserted through mesentery vein into portal vein and another catheter into the inferior vena cava. Pressure transducers (Philips CM 130) recorded PVP and IVCP. PVF was recorded with an electromagnetic flow meter (Nihonkoden). PVF, PVP and IVCP were checked four hours after injection and PVR was determined according to the formula: PVR= (PVP-IVCP)/PVF.

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PHT rats were divided into PVL, IPO model, and sham-operated groups, each group containing ten rats, and then divided at random into two groups of five rats. PVL, IPO and sham-operated rats were each intraperitoneally injected with LPS at the dose of 1.0 mg·kg⁻¹. The other PVL, IPO and sham-operated rats were intraperitoneally injected equivalent volumes of saline as control. PVF, PVP and IVCP were checked as above 4h after injection and PVR was determined according to the formula: \( \text{PVR} = (\text{PVP} - \text{IVCP}) / \text{PVF} \).

The alteration of portal hemodynamics of the noncirrhotic and sham-operated rats after injection of LPS was analyzed. The means and increment percentages of PVF, PVP, and PVR of the PVL and IPO groups were compared with that of the sham-operated group.

### Statistical analysis

Data were expressed as \( \bar{x} \pm s \). Statistical analysis between groups was made by means of the student’s unpaired \( t \) test by means of SPSS10.0 software, with \( P < 0.05 \) being regarded as statistically significant.

### RESULTS

#### Portal hemodynamic of model rats after operation

Just after portal vein ligation, PVF averaged 10.8 mL·min⁻¹, PVP increased to 1.85 kPa and PVR increased to 142 kPa·min⁻¹·L⁻¹. Two weeks after operation, PVF, PVP and PVR averaged 22.6 mL·min⁻¹, 1.86 kPa and 71 kPa·min⁻¹·L⁻¹. After finishing portal vein occlusion, PVF averaged 9.6 mL·min⁻¹, PVP increased to 2.05 kPa and PVR 180 kPa·min⁻¹·L⁻¹. Two weeks after operation, PVF, PVP and PVR averaged 22 mL·min⁻¹, 1.74 kPa and 67 kPa·min⁻¹·L⁻¹. PVP of the models was significantly increased the moment after operation and 2 wks after operation (\( P < 0.01 \)).

#### Effects of LPS on portal hemodynamic

PVF of all the groups of rats accepting intraperitoneal injection of LPS was significantly increased 4h after injection (\( P < 0.01 \)). Except for the group of rats accepting intraperitoneal injection of 0.1 mg·kg⁻¹ of LPS (\( P > 0.05 \)), the other groups of rats were all significantly increased in PVF 4h after injection (\( P < 0.01 \)). PVF and PVP increased in a dose-dependent manner with increasing LPS concentration. Except for the group of rats accepting intraperitoneal injection of 0.5 mg·kg⁻¹ of LPS (\( P > 0.05 \)), the other groups of rats were all decreased in PVR 4h after injection (\( P < 0.05 \)) and no dose-dependent manner of LPS was observed (Table 1).

#### Table 1  Effects of LPS on portal hemodynamics

| Dose of LPS (mg·kg⁻¹) | PVF (mL·min⁻¹) \( P \) | PVP (kPa) \( P \) | PVR (kPa·min⁻¹·L⁻¹) \( P \) |
|------------------------|--------------------------|-----------------|--------------------------|
| 0.00                   | 14.0±0.44                | 1.04±0.020      | 51                       |
| 0.10                   | 18.0±0.44                | 1.05±0.022      | 743                      | 42                       | 0.001                   |
| 0.25                   | 22.2±0.66                | 1.25±0.026      | 0.000                    | 44                       | 0.003                   |
| 0.50                   | 26.2±0.80                | 1.50±0.015      | 0.000                    | 48                       | 0.086                   |
| 1.00                   | 34.8±0.80                | 1.80±0.023      | 0.000                    | 45                       | 0.003                   |
| 2.00                   | 39.6±0.74                | 1.95±0.035      | 0.000                    | 44                       | 0.001                   |
| 4.00                   | 38.8±0.33                | 2.05±0.022      | 0.000                    | 47                       | 0.008                   |

Compare rats accepting intraperitoneal injection of various doses of LPS with rats not accepting LPS.

#### Effects of endotoxin on portal hemodynamic of PHT models

PVF and PVP of sham-operated rats increased from 14.0 mL·min⁻¹ and 1.04 kPa to 34.8 mL·min⁻¹ and 1.80 kPa. PVR decreased from 52 kPa·min⁻¹ to 44 kPa·min⁻¹ 4h after intraperitoneal injection of 1 mg·kg⁻¹ of LPS. PVF of PVL and IPO model rats increased to 32.8 mL·min⁻¹ and 28.0 mL·min⁻¹ respectively; PVP increased to 2.24 kPa and 1.95 kPa respectively; and PVR decreased to 61 kPa·min⁻¹ and 61 kPa·min⁻¹ respectively. In the three groups of rats, intraperitoneal injection 1 mg·kg⁻¹ of LPS significantly changed PVF, PVP and PVR (\( P < 0.01 \), Table 2).

The percentages of PVF increase in the PVL, IPO and sham-operated groups of rats were 45.1%, 27.3%, and 148.6% respectively. PVP increased 20.4%, 12.1%, and 73.1% respectively. PVR increased -14.1%, -9.0%, and -15.4% respectively (Table 3). The increase of PVF and PVP in the two groups of PHT model rats were significantly different from sham-operated rats (\( P < 0.01 \)). There was no significant difference between the decrease of PVR in the two groups of PHT model rats and sham-operated rats (\( P > 0.05 \) Table 3).

#### Table 2  Effects of LPS on portal hemodynamics of sham-operated and PHT rats

| Group                              | PVF (mL·min⁻¹) | PVP (kPa) | PVR (kPa·min⁻¹·L⁻¹) |
|------------------------------------|----------------|-----------|---------------------|
| Portal vein ligation               | 32.8±1.6       | 2.24±0.073| 61                  |
| Control                            | 22.6±1.7       | 1.86±0.044| 71                  |
| Intrahepatic portal occlusion      | 28.0±2.1       | 1.95±0.054| 61                  |
| Control                            | 22.0±2.1       | 1.74±0.037| 61                  |
| Sham-operated                      | 34.8±0.7       | 1.80±0.046| 44                  |
| Control                            | 14.0±0.4       | 1.04±0.039| 52                  |

#### Table 3  Alteration of portal hemodynamics of the noncirrhotic and sham-operated rats after injection of LPS

| Group                              | PVF (mL·min⁻¹) | PVP (kPa) | PVR (kPa·min⁻¹·L⁻¹) |
|------------------------------------|----------------|-----------|---------------------|
| PVF                                | 10.2±0.8       | 45.13     | 0.38±0.047          | 20.43 | -10 | -14.08 |
| IPO                                | 6.4±1.14       | 27.27     | 0.21±0.026          | 12.07 | -10 | -8.96  |
| Sham-operated                      | 20.8±0.8       | 148.57    | 0.76±0.038          | 73.08 | -8  | -15.38 |

#### DISCUSSION

Portal hypertension (PHT) is mainly due to two factors, PVF and PVP. Increase of PVF could lead to portal congestion, and PVP could prevent portal output and lead to portal gore. PHT is apt to be associated with a series of cytokines and vasodilators[29]. Endotoxin could enhance synthesis of a series of vasoconstrictors such as endothelins, as well as a series of vasodilators such as nitric oxide (NO). These modulators are able to adjust portal and systemic hemodynamics functionally. Across the cell’s membrane, NO could spread to smooth muscle cells, enhance synthesis of cyclic guanosine monophosphate (cGMP), and consequently decrease intracellular Ca²⁺ concentrations, thus inducing vasorelaxation[30]. NO could also increase cardiac output and lower the vessel’s reaction to vasoconstrictors, causing systemic and splanchnic hyperdynamic circulation[20,21]. Our research proved LPS could increase PVF of normal and noncirrhotic portal hypertensive rats and that this increase was associated with the dosage of LPS, which demonstrated increasing PVP was an important factor to form PHT. Endotoxemia could modulate the intrahepatic portal vessel and consequently alter the resistance of the intrahepatic portal vessel[22, 23]. Endotoxin signals hepatic cells to secret a series of cytokines such as tumor necrosis factor (TNF-α) and endothelin and consequently enhances synthesis and deposition of collagen[25-27]. Endothelin has been reported to be able to induce constriction of the smooth muscle cells of the hepatic vasculature[29]. Endothelin can also prompt hepatic stellate cells (HSC) to proliferate and constrict[28]. Endotoxin was thought to increase PVF by the ways above. However, endotoxin-induced increase of NO synthesized by inducible nitric oxide synthase could lead to vasorelaxation and lower the vessel’s response to vasoconstrictors, which might account for the increase of PVR. Yokoyama reported the liver maintains its microcirculatory flow by vascular remodeling from the hepatic arterial vasculature following PVL[30], which might induce the decrease of PVR in noncirrhotic
PHT rats. This research shows the PVR of normal and noncirrhotic PHT rats decreased after intraperitoneal injection of LPS, which demonstrated effectively that increasing PVF was the main factor to forming PHT.

PHT models moderate PVP through a new balance of vas constrictorists and vasodilators. PHT model rats were reported to be sensitive to LPS by means of portal vein ligation. But Chu suggested some evidence against a role for endotoxin in the hyperdynamic circulation of rats with prehepatic portal hypertension. Our experiments show that after intraperitoneal injection of LPS, PVF and PVP of PVL and IPO model rats increased significantly less than that of sham-operated rats (P<0.01). Another report found artery vessel of PVL rats more blunt to LPS and the increment of NOS was significantly reduced tumour necrosis factor alpha production.

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