Effect of intestinal lymphatic circulation blockage in two-hit rats

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Abstract

AIM: To study the effect of blocking intestinal lymphatic circulation in two-hit rats and explore the significance of intestinal lymphatic circulation in two-hit.

METHODS: Wistar rats were divided equally into three groups: mesenteric lymph duct ligation group, non-ligation group and sham group. Mesenteric lymph was diverted by ligation of mesenteric lymph duct, and the two-hit model was established by hemorrhage and lipopolysaccharide (LPS) methods. All rats were sampled for serum pre-experiment and 24 h post-experiment. The organs including kidney, liver, lung and heart were collected for pathomorphologic observation and biochemical investigation. The nitric oxide (NO), malondialdehyde (MDA) and superoxide dismutase (SOD) were determined in serum and tissue homogenate.

RESULTS: Pathomorphology study showed that the structures of kidney, lung, liver and heart tissues were normal in sham group; congestion, degeneration and necrosis in non-ligation group; but only mild lesions in ligation group. After two-hits, the contents of AST, ALT, BUN, Cr and LDH-1 in the serum of non-ligation group and ligation group were obviously higher than that in pre-experiment group and sham group, but obviously lower than that in non-ligation group. The contents of NO_2^-/NO_3^-, NOS, iNOS and MDA in the serum of non-ligation group were significantly increased, compared with pre-experiment and sham group, but SOD was significantly lower. These parameters were significantly different in ligation group compared with that in sham group, but NO_2^-/NO_3^-, iNOS and MDA in ligation group were significantly lower than that in non-ligation group.

CONCLUSION: Ligation of mesenteric lymph duct could improve the disturbance of organic function and morphologic damage in two-hit rats; the lymphatic mechanism in two-hit should be emphasized.

INTRODUCTION

Multiple organ dysfunction syndrome (MODS) is a common but poorly understood complication in a variety of critical illnesses[1-3]. In recent years, much attention has been paid to the study of MODS, and encouraging progress has been achieved. It has been accepted that the lymph system has an independent function and significance, which is different from the traditional concept that it is an assistant system of organic fluid circumfluence. In various pathogenesis of MODS, the complicated net of inflammatory mediators and cytokines is increasingly noticed. It has always been believed that their transport and transmit are *via* blood[4-6]. However, whether the lymphatic pathway is meaningful to pathogenesis of MODS is yet to be clarified. We previously showed that lymph microcirculation is closely related to the occurrence and development of shock[7,8]. Deitch *et al*[9-12] reported that mesenteric lymph could activate neutrophils *in vivo* and *ex vivo*, promote the development of acute lung injury after resuscitation of hemorrhagic shock; when mesenteric lymph duct was ligated in advance, lung injury could be avoided. In the present study, in order to disclose the pathogenic importance of mesenteric lymph in MODS, the two-hit model was duplicated by two-hits of hemorrhage/resuscitation and lipopolysaccharide (LPS); the protective effect of intestinal lymphatic circulation block on organs in two-hit rats was investigated, and the influence of mesenteric lymph duct ligation on nitric oxide (NO) and free radicals in two-hit rats was explored.

MATERIALS AND METHODS

Animals

Forty-five male Wistar rats weighing 280 to 350 g (supplied by the Experimental Animal Center of Hebei Medical University), were divided equally into three groups: mesenteric lymph duct ligation group, non-ligation group and sham group.

Duplication of two-hit model

Rats were generally anesthetized with pentobarbital sodium (50 mg/kg) by intramuscular injection. A median incision was made in nuchae. The right common carotid arteries and left jugular veins were dissected, and cannulated to facilitate the blood withdrawal and infusion resuscitation. The two-hit model was duplicated by modified Spain's method[13]. Blood (one sixth of body blood volume and one thirteenth of avoirdupois) was drawn by the automatic withdrawal-infusion machine (type of ZCZ-50) until the mean arterial pressure fell to a low level (less than 50 mmHg). The rate in the beginning was 0.4 mL/min for 5 min, then uniform velocity maintained at 0.1 mL/min. The process of blood withdrawal lasted more than 20 min and the blood was stored for serum tests. After being hypotensive for 40 min, the rats of both the ligation group and non-ligation group were resuscitated by Ringer's solution for as much as 3 times the lost blood volume for more than 30 min, then ligated through the right common carotid arteries and left jugular veins, and the incisions were then sutured. All the rats of the three groups received an abdominal operation with a vertical incision of 4 cm in length. The end of the mesentery was exposed. The mesenteric lymphatic ducts, which ran along the superior mesenteric artery, were separated. The rats of the ligation groups were additionally ligated at the mesenteric lymph duct, whereas sham and non-ligation groups were not, these were only threaded with cotton below the mesenteric lymph duct and then the abdomen was closed. After 6 h, LPS (O111:B4, Sigma) was injected intraperitoneally at a dose of 4 mg/kg, and 5% glucose saline at 20 mL/kg every 2 h until after 22 h for supportive therapy. After 24 h, all rats were anesthetized again with sodium pentobarbital (25 mg/kg), and their left common carotid arteries were cannulated to sample blood for serum tests by centrifugation. Then all rats were sacrificed and the organs were taken out, including the kidney, liver, lung and heart. Microscopic sections were made. The sera and tissue homogenate were stored at -26℃ for later use.

Biochemical indexes and pathomorphology

The biochemical indexes of hepatic and renal function and myocardiac enzymes including the aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (Cr), lactic acid dehydrogenase-1 (LDH-1) were determined before and after experimentation by an automatic biochemical analyzer type of Aeroset. The kidney, liver, lung and heart tissues were fixed by formaldehyde, embedded in paraffin, sliced and stained by HE. The pathological changes of the tissues were observed.

Determination of NO_2^-/NO_3^-, NOS, SOD and MDA in serum and tissue homogenate

The concentrations of NO_2^-/NO_3^-, nitric oxide synthase (NOS), inducible nitric oxide synthase (iNOS), superoxide dismutase (SOD) and malondialdehyde (MDA) in serum and tissue homogenate were determined by nitrate reductase method, chemical chromogenic reaction, modified TBA microdetermination, xanthinoxidase method (Naijing Jiancheng, China).

Statistical analysis

All data were expressed as mean ± SD. SPSS software (Version 11.0 for Windows 98) was employed, one-way analysis of variance was used between groups, paired t test within group, and χ^2 test for survival analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Survival rate of two-hit rats

In the process of the two-hit for 24 h, the survival rates of rats in the sham group, non-ligation group and ligation group were 100% (15/15), 53.3% (8/15), 73.3% (11/15) respectively, with a significant difference between the three groups ($\chi^2 = 8.904$, $P < 0.05$).

Effect of mesenteric lymph duct ligation on biochemical indexes in two-hit rats

There was no obvious difference between the three groups in the serum biochemical indexes of liver, kidney and cardiac muscle function before experimentation, as well as before and after experimentation in the sham group. The levels of AST, ALT, BUN, Cr and LDH-1 in the ligation group and non-ligation group were obviously higher than that in the sham group after experimentation, as well as before experimentation ($P < 0.01$, $P < 0.05$). The levels of ALT, BUN and Cr in the ligation group were obviously lower than that in non-ligation group ($P < 0.01$, Table 1).

Pathomorphologic changes of the vital organs

Kidney: The structure of the glomerulus and renal tubule, proximal and distal convoluted tubes were seen clearly in the sham group (Figure 1A). Fibrinoid necrosis of capillary vessels of the glomerulus, plasma protein precipitation in the glomerular capsule, and necrosis in some renal tubules could be observed in the non-ligation group (Figure 1B). There was fibrinoid necrosis on the capillary wall of the glomerulus in the ligation group, but only a few protein casts in the renal tubule (Figure 1C).

Lung: In the sham group, the structure of the alveolus was normal. The alveolar wall was thin. There were alveolar epithelial cells on the surface. Capillary vessels of the alveolar wall were not dilated or congested. There was no exudate in the alveolar cavity (Figure 2A). In the non-ligation group, severe monopyrenous cell hyperplasia and hemorrhage in the alveolar gap could be seen, leading to atelectasis and some alveolar emphysematous change (Figure 2B). In the ligation group, there were hyperplasia of monopyrenous cells and hemorrhage in the alveolar

Table 1 Effect of mesenteric lymph duct ligation on biochemical indexes of two-hit rats (mean ± SD)

Group	*n*		AST (nkat/L)		ALT (nkat/L)		BUN (mmol/L)		Cr (μmol/L)		LDH-1 (nkat/L)	
	Pre-exp	Post-exp	Pre-exp	Post-exp	Pre-exp	Post-exp	Pre-exp	Post-exp	Pre-exp	Post-exp	Pre-exp	Post-exp
Sham	15	15	1767 ± 333	1917 ± 550	533 ± 150	583 ± 283	7.4 ± 1.0	8.5 ± 1.6	47.7 ± 22.1	50.9 ± 31.7	4334 ± 2033	4051 ± 2567
Non-ligation	15	8	1817 ± 633	5801 ± 1900[b,d]	550 ± 133	4117 ± 1450[b,d]	7.5 ± 0.9	21.8 ± 3.5[b,d]	47.5 ± 18.9	213.9 ± 81.2[b,d,f]	4017 ± 2017	7985 ± 2267[b,d]
Ligation	15	11	1667 ± 483	5184 ± 2300[b,d]	500 ± 167	2967 ± 1217[b,d,f]	7.8 ± 1.1	15.5 ± 5.6[b,d,f]	43.3 ± 18.4	142.0 ± 66.1[a,b,d]	4917 ± 1767	7151 ± 2250[b,d]

[b]$P < 0.01$ *vs* pre-exp; [d]$P < 0.01$ *vs* sham; [a]$P < 0.05$, [f]$P < 0.01$ *vs* non-ligation.

Figure 1 Effect of mesenteric lymph duct ligation on pathomorphology of kidney in MODS rats (HE × 200). **A**: Sham; **B**: Non-ligation; **C**: Ligation.

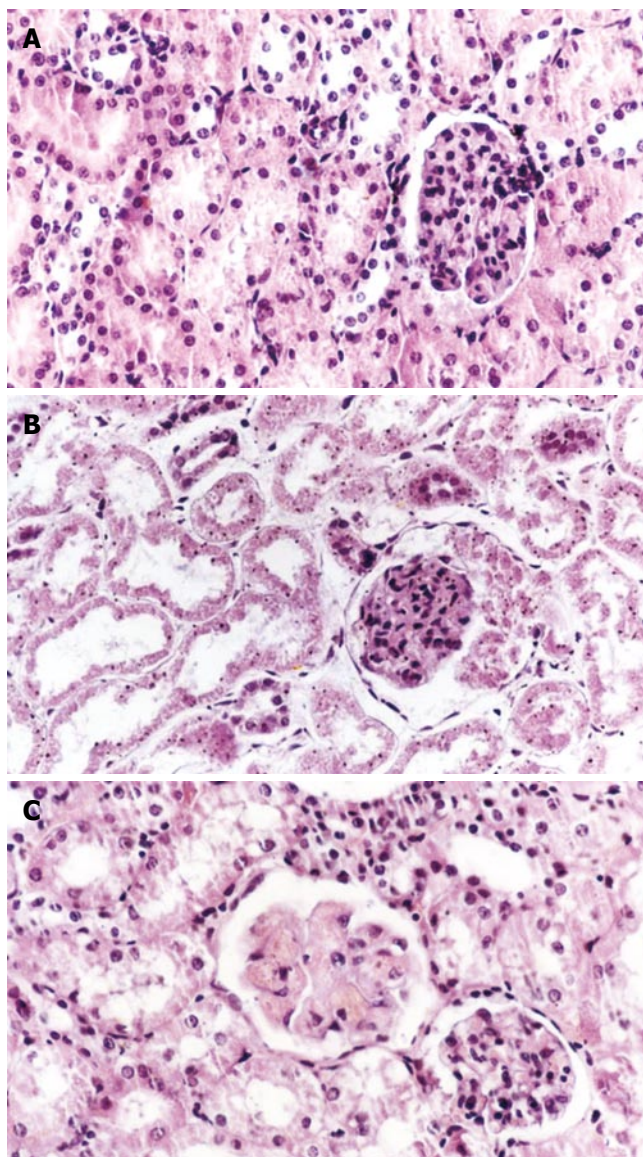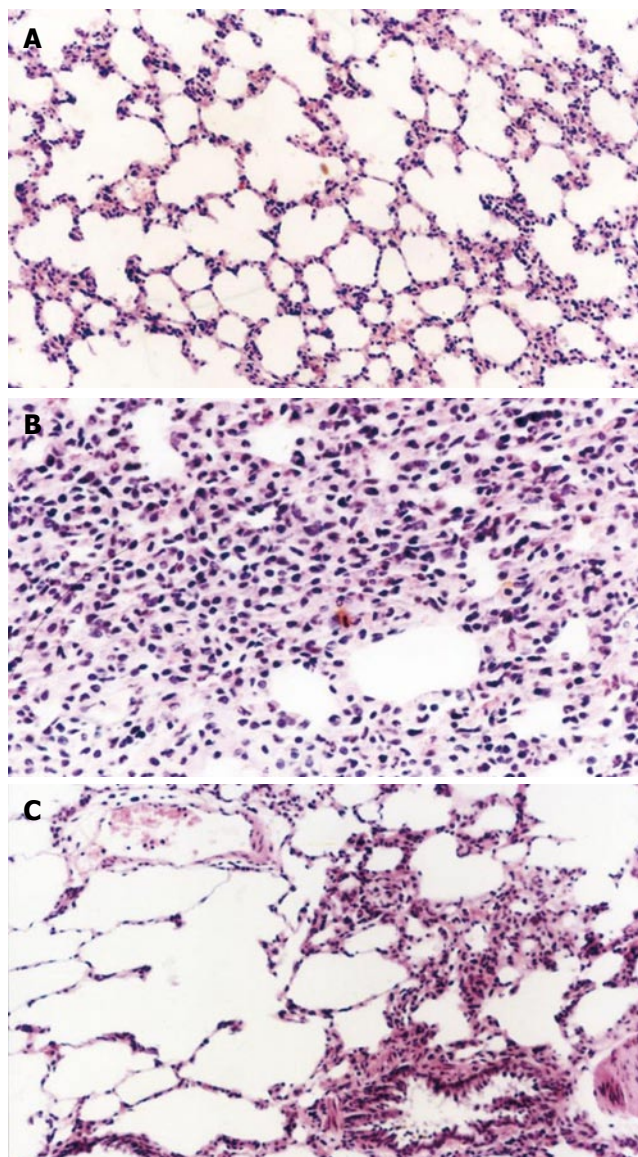**Figure 2** Effect of mesenteric lymph duct ligation on pathomorphology of lung in MODS rats (HE × 100). **A**: Sham; **B**: Non-ligation; **C**: Ligation.

gap, resulting in widening of the alveoar gap and atrophy of alveoli. The surrounding alveoli were compensatorily emphysematous and the alveolar walls were thinner and broken. The local blood vessels were dilated and hematose (Figure 2C).

Liver: In the sham group, the central vein of the liver lobule was dilated slightly. The liver cells were arranged slightly disordered, but all were of the same size.

Their nucleoli were round and centrally located. Their karyotheca was clear (Figure 3A). In the non-ligation group, the liver cells were necrotic, with karyopycnosis, karyorrhexis, karyolysis and focal hemorrhage (Figure 3B). In the ligation group, the central vein of the liver lobule and the surrounding liver sinuses were obviously dilated and hematose. Liver cells arranged disorderly, but their conformations were normal (Nucleoli were of the same
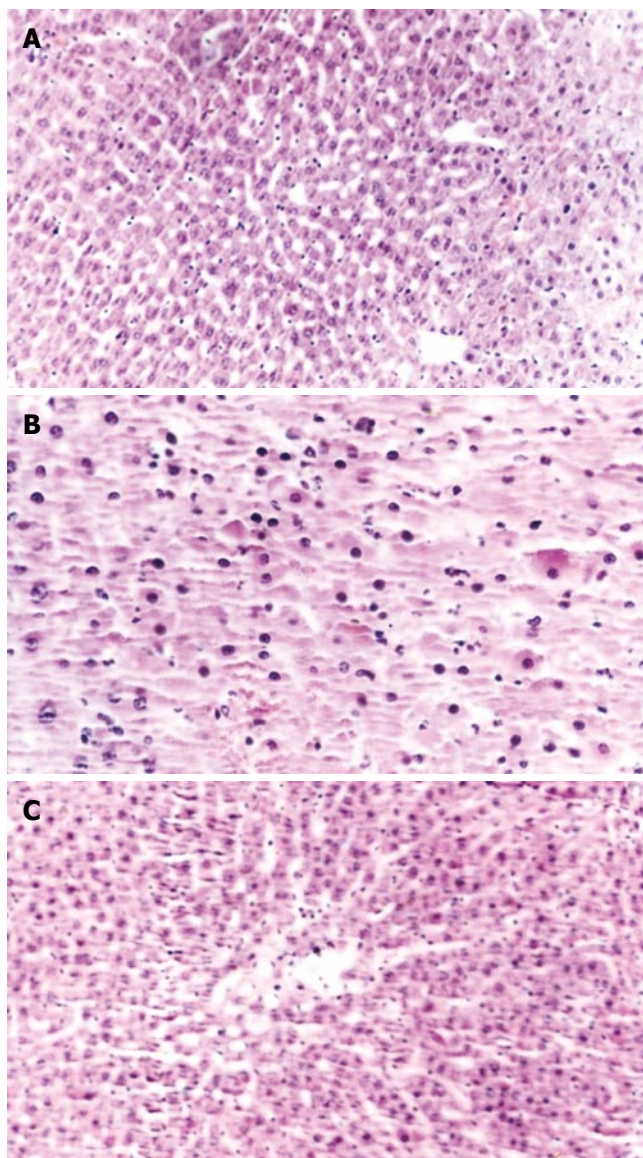
Figure 3 Effect of mesenteric lymph duct ligation on pathomorphology of liver in MODS rats (HE × 100). **A**: Sham; **B**: Non-ligation; **C**: Ligation.

Figure 4 Effect of mesenteric lymph duct ligation on pathomorphology of heart in MODS rats (HE × 200). **A**: Sham; **B**: Non-ligation; **C**: Ligation.

size, round and centered. The karyotheca was clear.). Only some liver cells were necrotic, such as karyopycnosis, karyorrhexis and karyolysis (Figure 3C).

Cardiac muscle: In the sham group, the structure of the cardiac muscle fiber was normal. Cell nucleolus was centered, karyotheca was clear, and cardiac muscle cells were of the same size (Figure 4A). In the non-ligation group, there were coagulation necroses in the cardiac muscle fiber. Only the nucleolus of the myocardial mesenchyme was left, with focal sarcoplasm lysis and necrosis. A few structures of mesenchyme were seen (Figure 4B). In the ligation group, there were focal sarcoplasm coagulations in the cardiac muscle fiber. The nucleolus disappeared and the myocardial mesenchyme had focal infiltration of inflammatory cells (Figure 4C).

Effects of mesenteric lymph duct ligation on NO content and NOS activity in two-hit rats

Effects on NO content and NOS activity in serum: The contents of NO_2^-/NO_3^-, NOS and iNOS in serum

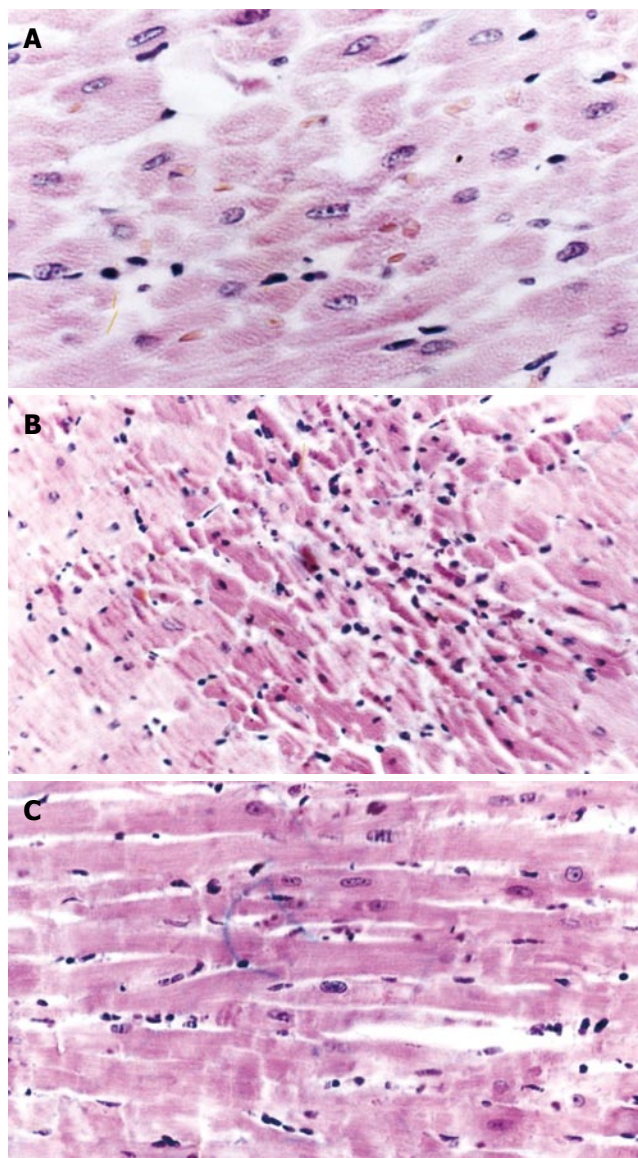had no significant difference between the three groups before experimentation and in the sham group between pre- and post-experimentation. After two-hit, the contents of NO_2^-/NO_3^-, NOS and iNOS in the serum of the non-ligation group were significantly higher than that before experimentation and in the sham group ($P < 0.01$). The contents of NO_2^-/NO_3^- and NOS of the ligation group were significantly higher than that in the sham group ($P < 0.05$), but were significantly lower than in the non-ligation group ($P < 0.01$, Table 2).

Effects on NO content and NOS activity in tissue homogenate: Compared with the sham group, the contents of NO_2^-/NO_3^- in intestine, kidney, liver, lung and heart homogenates in the non-ligation group were significantly increased ($P < 0.01$), as well as in kidney, lung and heart homogenate in the ligation group, but no significant difference existed in intestine and liver ($P > 0.05$). The contents of NO_2^-/NO_3^- in intestine, kindey and liver homogenate of the ligation group were significantly lower than that of the non-ligation group ($P < 0.01$), but

Table 2 Effect of mesenteric lymph duct ligation on NO$_2^-$/NO$_3^-$, NOS and iNOS in serum of two-hit rats (mean ± SD)

Group	n		NO$_2^-$/NO$_3^-$ (mmol/L)		NOS (nkat/L)		iNOS (nkat/L)	
	Pre-exp	Post-exp	Pre-exp	Post-exp	Pre-exp	Post-exp	Pre-exp	Post-exp
Sham	15	15	84.2 ± 48.4	79.4 ± 33.3	245 382 ± 132 026	219 544 ± 78 349	87 851 ± 43 842	71 681 ± 37 647
Non-ligation	15	8	88.6 ± 38.5	217.1 ± 48.1[b,d]	251 550 ± 69 347	389 411 ± 90 851[b,d]	91 685 ± 22 338	175 035 ± 45 009[b,d]
Ligation	15	11	86.3 ± 41.7	159.9 ± 44.1[b,d,f]	265 053 ± 102 520	312 562 ± 129 193[a]	85 017 ± 42 842	109 355 ± 24 005[f]

[b]*P* < 0.01 *vs* pre-exp; [a]*P* < 0.05, [d]*P* < 0.01 *vs* sham; [f]*P* < 0.01 *vs* non-ligation.

Table 3 Effect of mesenteric lymph duct ligation on NO$_2^-$/NO$_3^-$, NOS and iNOS in homogenate of two-hit rats (mean ± SD)

	Group (n)	Intestine	Kidney	Liver	Lung	Heart
NO$_2^-$/NO$_3^-$ (μmol/g)	Sham (15)	3.3 ± 1.0	1.4 ± 0.3	1.6 ± 1.0	1.4 ± 0.5	2.0 ± 0.4
	Non-ligation (8)	9.1 ± 2.9[b]	5.1 ± 1.4[b]	4.0 ± 1.2[b]	3.2 ± 0.9[b]	3.5 ± 0.9[b]
	Ligation (11)	4.3 ± 2.2[d]	2.9 ± 0.7[b,d]	2.2 ± 0.8[d]	2.7 ± 0.7[a]	3.1 ± 0.6[a]
NOS (nkat/g)	Sham (15)	20.7 ± 4.7	11.2 ± 4.5	12.2 ± 4.3	0.62 ± 0.17	24.8 ± 3.7
	Non-ligation (8)	30.2 ± 9.7[a]	15.5 ± 6.3[b]	18.8 ± 8.2	0.84 ± 0.32	28.5 ± 5.1
	Ligation (11)	27.0 ± 6.8	13.7 ± 4.5	13.5 ± 3.8	0.73 ± 0.15	26.5 ± 5.1
iNOS (nkat/g)	Sham (15)	9.8 ± 2.2	6.2 ± 2.8	5.8 ± 2.8	4.0 ± 1.0	11.0 ± 4.0
	Non-ligation (8)	15.3 ± 3.8[b]	7.7 ± 3.3	8.0 ± 5.0	7.0 ± 3.2	15.2 ± 6.0
	Ligation (11)	12.0 ± 2.3	7.0 ± 4.0	7.0 ± 3.5	5.7 ± 1.3	12.0 ± 4.0

[a]*P* < 0.05, [b]*P* < 0.01 *vs* sham; [d]*P* < 0.01 *vs* non-ligation.

Table 4 Effect of mesenteric lymph duct ligation on SOD and MDA in serum of two-hit rats (mean ± SD)

Group	n		SOD (nkat/L)		MDA (μmol/L)	
	Pre-exp	Post-exp	Pre-exp	Post-exp	Pre-exp	Post-exp
Sham	15	15	1 732 013 ± 134 027	1 687 004 ± 100 020	6.01 ± 1.53	6.82 ± 1.17
Non-ligation	15	8	1 711 342 ± 139 195	1 394 446 ± 210 542[b,d]	5.55 ± 1.18	10.06 ± 1.28[b,d]
Ligation	15	11	1 664 666 ± 116 690	1 610 489 ± 149 197[f]	6.46 ± 1.48	8.52 ± 1.16[b,d,f]

[b]*P* < 0.01 *vs* Pre-exp; [d]*P* < 0.01 *vs* sham; [f]*P* < 0.01 *vs* non-ligation.

lung and heart homogenate had no significant difference between the two groups (*P* > 0.05). The NOS activity in intestine and kidney homogenate of the non-ligation group was significantly increased compared with the sham group (*P* < 0.01, *P* < 0.05), and that in liver, lung and heart homogenate seemed to be increased, but with no significant difference (*P* > 0.05). The iNOS activity in intestine homogenate of the non-ligation group was higher than that of the sham group (*P* < 0.01), but kidney, liver, lung and heart homogenate had no significant difference (*P* > 0.05). The NOS and iNOS activity in intestine, kidney, liver, lung and heart homogenate of the ligation group had no significant difference compared with the sham group and the non-ligation group (*P* > 0.05, Table 3).

Effects of mesenteric lymph duct ligation on SOD activity and MDA content in two-hit rats

Effects on SOD activity and MDA content in serum: The SOD activity and MDA content in serum had no significant difference in the three groups before experimentation and in the sham group pre- and post-experimentation (*P* > 0.05). After experimentation, the MDA content of serum in the non-ligation group and ligation group were significantly increased than that of pre-experimentation and in the sham group (*P* < 0.01), but the MDA content in the ligation group was significantly lower than that of the non-ligation group (*P* < 0.01). The SOD activity in the non-ligation group was significantly lower than that of pre-experimentation, sham group and ligation group (*P* < 0.01), but SOD activity in the ligation group had no significant difference from pre-experimentation and in the sham group (*P* > 0.05, Table 4).

Effects on SOD activity and MDA content in tissue homogenate: The SOD activity of intestine homogenate in non-ligation group was significantly lower than that in sham group (*P* < 0.05), but SOD activity of intestine and heart in ligation group was higher than that in non-ligation group (*P* < 0.05), whereas in other tissue homogenates it had no significant difference (*P* > 0.05). The MDA content of intestine, kidney and liver homogenate in the non-

Table 5 Effect of mesenteric lymph duct ligation on SOD and MDA in homogenate of two-hit rats (mean ± SD)

Group (n)		Intestine	Kidney	Liver	Lung	Heart
SOD (nkat/g)	Sham (15)	442 255 ± 77 349	273 221 ± 43 009	205 374 ± 35 840	226 212 ± 38 174	300 227 ± 58 011
	Non-ligation (8)	330 399 ± 77 516[a]	268 720 ± 20 504	204 874 ± 20 838	175 035 ± 44 676	241 882 ± 56 178
	Ligation (11)	467 594 ± 74 182[c]	256 885 ± 28 172	212 376 ± 35 674	192 205 ± 47 009	339 235 ± 49 510[c]
MDA (μmol/g)	Sham (15)	2.26 ± 0.65	1.62 ± 0.45	1.66 ± 0.52	1.12 ± 0.25	1.33 ± 0.36
	Non-ligation (8)	3.28 ± 0.58[a]	2.28 ± 0.16[a]	2.27 ± 0.28[a]	1.34 ± 0.33	1.84 ± 0.62
	Ligation (11)	2.81 ± 0.59	2.22 ± 0.58[a]	2.24 ± 0.34[a]	1.32 ± 0.20	1.52 ± 0.40

[a]$P < 0.05$ vs sham; [c]$P < 0.05$ vs non-ligation.

ligation group and of kidney and liver homogenate in the ligation group were higher than that in the sham group ($P < 0.05$). The MDA content of lung and heart homogenate in non-ligation and of intestine, lung, heart homogenate in the ligation group had no significant difference from those of the sham group ($P > 0.05$), and no significant difference existed between the non-ligation group and the ligation group, either ($P > 0.05$, Table 5).

DISCUSSION

The occurrence of MODS is related to the pathogenic causes such as trauma, shock, ischemia-reperfusion injury and endotoxemia, etc[14]. The various inflammatory cells are continuously stimulated and activated by pathogenic factors, producing and releasing mass cytokines and inflammatory mediators. There are complex chain reactions and cytokine networks between cytokines, cytokine and inflammatory mediators[12,15]. In the process of production and release, they stimulate each other, act synergistically, or inhibit and counteract with each other, producing a cytokine cascade effect, and gradually an amplified effect on every organ and system, leading to the non-specific injury of tissues, damage of organs, and finally the occurrence of systemic inflammatory response syndrome (SIRS)[1,16-19]. This is also the important pathophysiologic mechanism causing MODS. The MODS model can be made by one-hit and two-hit methods[13]. In our study, the MODS rat model was successfully established by a two-hit method of hemorrhage/resuscitation and LPS, and the two-hit model showed different degrees of damage and dysfunction of liver, kidney and myocardium from pathomorphology and biochemical indexes of hepatic, renal function and myocardial enzyme at pre- and post-experimentation. Through this model, the effect of blocking intestinal lymphatic circulation in two-hit rats was studied.

In pathogenesis of diphase type MODS, damage of the intestinal barrier function would induce intestinal endotoxemia and bacterial translocation from intestine[14,21]. The circumfluence of intestinal and celiac contents has both blood and lymph routes[22]. We investigated the importance of intestinal mesenteric lymph circumfluence in MODS pathogenesis in two-hit rats by ligating mesenteric lymphatic duct. The biochemical indexes of liver, kidney, and heart function before and after two-hit in three groups showed that after two-hit, the contents of AST, ALT, BUN, Cr and LDH-1 in the serum of the non-

ligation group and ligation group were obviously higher than that before experimentation and in the sham group. The contents of ALT, BUN and Cr in the ligation group were obviously lower than in the non-ligation group. All these suggest that the ligation of mesenteric lymph duct had a protective effect on the function of liver, kidney and heart because the ligation led to a decrease of the entry of intestinal bacteria and endotoxins into blood. A pathomorphologic study showed that the cellular structures of the kidney, lung, liver and heart tissues in the sham group were normal, while congestion, degeneration and necrosis were found in organs of the non-ligation group, and only mild lesions could be found in the ligation group. It suggests that the ligation of mesenteric lymph duct had a protective effect on the liver, kidney, lung, and heart in two-hit rats. In addition, ligating mesenteric lymph duct could help resuscitation from two hits of hemorrhage and LPS, improve the survival rate; meanwhile it also confirmed the protective effects of mesenteric lymph diversion.

After LPS was injected into the abdominal cavity of the wounded rats, the histology of lymphatic tissue showed that there existed a lymphatic micronet at the visceral and parietal peritoneum; the largest surface area was those covering the digestive tube. These lymphatic capillary nets formed the lymphatic plexus, which sent out collecting lymphatic vessels to regional nodes. Mesenteric lymph duct is a chief excurrent passage. When the function of intestinal barrier was damaged, the toxic substance also could be translocated through mesenteric lymph duct[23]. By ligation of mesenteric lymph duct, the blockage of the lymph stream from intestines and peritoneum would make LPS (MW = 30 kD) and other intestinal poisonous substances difficult to get into mesenteric lymph duct, thus relieving the morphologic damage and functional disorder of many organs.

Intestinal barrier dysfunction and intestinal inflammation caused by bacteria/endotoxin translocation (BET) are the main reason for pyotoxinemia and MODS in severe inflammatory patients with unclear origin[24-27]. In recent years[28,29], some scholars also put forward that the intestines are the "center" organ for MODS and the chief cell origin for TNFα. Thus the intestines are not only the target organs of damage, but also the main links in MODS. In our experiment, probably via blocking the linkage function of the intestines as center organs, ligation of mesenteric lymph duct exerted the protective effect on several organs. From the contents of NO, NOS, iNOS

and MDA in serum and tissue homogenate, it indicates that the cytokine cascade reaction was induced by two-hits of hemorrhage and LPS. It also suggests that ligation could reduce iNOS, NOS, NO and free radicals produced and released by the intestines into the systemic circulation through lymph ducts, thus alleviating their damage to the organs.

LPS is a main component of the outer cell wall structure of Gram-negative bacteria. Although micro-lymph ducts have the function of absorbing macromolecular substances actively, in this experiment, LPS was injected into the abdominal cavity, but not the digestive cavity directly. Therefore, the majority of LPS not only could be absorbed *via* the visceral peritoneum of the digestive tube which has the largest area, but also could be absorbed *via* micro-lymph ducts of other visceral peritoneum, then through lymphatic plexus which send out collecting lymphatic vessels to regional nodes, or through microvessels into blood. This might be the reason for high MDA content in liver and kidney homogenate of the ligation group, which was significantly higher than that of the sham group, but not significantly different from the non-ligation group.

In conclusion, ligation of mesenteric lymph duct has a protective effect in two-hit rats. It could reduce the production of iNOS, synthesis of NO, release of free radicals, and consumption of SOD, thus improving the disturbance of organic function and pathomorphological changes, and relieving the damage of two-hits on kidney, liver, lung, heart and intestine. It indicates that mesenteric lymph plays an important role in cytokine production and transportation of inflammatory response mediators in two-hit rats. Blockade of mesenteric lymph might be a new approach in prevention and treatment of two-hits.

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