Relationship between plasma D(-)-lactate and intestinal damage after severe injuries in rats

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Project supported by the Fund for National Outstanding Young Researchers of China, No. 39525024
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Received 2001-04-06 Accepted 2001-05-12

Abstract
AIM To explore the kinetic changes in plasma D(-)-lactate and lipopolysaccharide (LPS) levels, and investigate whether D(-)-lactate could be used as a marker of intestinal injury in rats following gut ischemia/reperfusion, burn, and acute necrotizing pancreatitis (ANP).

METHODS Three models were developed in rats: ¹gut ischemia/reperfusion obtained by one hour of superior mesenteric artery occlusion followed by reperfusion; ²severe burn injury created by 30% of total body surface area (TBSA) full-thickness scald burn; and ³ANP induced by continuous inverse infusion of sodium taurocholate and trypsin into main pancreatic duct. Plasma levels of D(-)-lactate in systemic circulation and LPS in portal circulation were measured by enzymatic-spectrophotometric method and limulus amebocyte lysate (LAL) test kit, respectively. Tissue samples of intestine were taken for histological analysis.

RESULTS One hour gut ischemia followed by reperfusion injuries resulted in a significant elevation in plasma D(-)-lactate and LPS levels, and there was a significant correlation between the plasma D(-)-lactate and LPS (r = 0.719, P<0.05). The plasma concentrations of D(-)-lactate and LPS increased significantly at 6h postburn, and there was also a remarkable correlation between them (r = 0.877, P<0.01). D(-)-lactate and LPS levels elevated significantly at 2h after ANP, with a similar significant correlation between the two levels (r =0.798, P<0.01). The desquamation of intestine villi and infiltration of inflammatory cells in the lamina propria were observed in all groups.

CONCLUSION The changes of plasma D(-)-lactate levels in systemic blood paralleled with LPS levels in the portal vein blood. The measurement of plasma D(-)-lactate level may be a useful marker to assess the intestinal injury and to monitor an increase of intestinal permeability and endotoxemia following severe injuries in early stage.

Subject headings gut/injury; ischemia reperfusion; blood; burn/blood; acute necrotizing pancreatitis/blood; D (-)-lactate/blood; lipopolysaccharide/blood; intestinal permeability

Sun XQ, Fu XB, Zhang R, Lü Y, Deng Q, Jiang XG, Sheng ZY. Relationship between plasma D(-)-lactate and intestinal damage after severe injuries in rats. World J Gastroenterol, 2001;7(4):555-558

INTRODUCTION
Apart from their major functions of digestion and absorption of nutrients, the intestines also act as a barrier to prevent micro-organisms and toxins contained within the lumen from spreading to distant tissues and organs[8-12]. Failure of intestinal barrier function often occurs in many clinical conditions, including hemorrhage shock, severe burn injury, and the surgically critical illness, resulting in the increased intestinal permeability and subsequent translocation of bacteria or/endotoxin from gut[13-20]. It is clear that increased gut permeability and bacteria with or without endotoxin translocation play a key role in the development of severe complications such as systemic inflammatory response syndrome (SIRS), sepsis, multiple organ dysfunction syndrome (MODS) and multiple organ failure (MOF)[21,22]. D(-)-lactate is a product of bacterial fermentation. It is produced by many of the bacteria found in the human gastrointestinal tract[23]. Tissues in mammalian do not produce it and its metabolism is very slow[24]. In this study, we investigated the changes of plasma D(-)-lactate and lipopolysaccharide (LPS) levels and their correlation in gut ischemia/reperfusion, burn injury and acute necrotizing pancreatitis (ANP); and explored whether the changes of D(-)-lactate levels could be used as a predictor of increased intestinal permeability and endotoxemia following severe injuries.

MATERIALS AND METHODS
Animals
Male Wistar rats were used in this serial studies. They were housed in individual cages. The room temperature was maintained at 22°C - 24°C with a 12h light-dark cycle, and free access to a commercial laboratory rodent chow and fresh water were allowed. Twelve hours prior to experiment, the rats were fasted, but allowed free access to water.

Rat models of gut ischemia/reperfusion
Rats weighing 190g - 250g were divided into three groups. Gut ischemic group (n = 20): Animals were anesthetized with an intraperitoneal injection of 0.3mL 30g·L⁻¹ pentobarbital sodium. Through a middle abdominal incision, intestinal...
ischemia was produced by occluding the superior mesenteric artery for 1 and 1.5h with an automatic microvascular clamp. Animals were sacrificed at the end of gut ischaemia. Gut ischemia/reperfusion group (n=50): superior mesenteric artery was occluded for 1h and then the vascular clamp was removed to produce gut reperfusion. Animals were sacrificed at 0.5, 1, 2, 6 and 24h after gut reperfusion. Sham-operated control (n=10): animals were treated identically omitting the superior mesenteric artery occlusion. Blood samples were collected aseptically from cervical artery and portal vein for D (-)-lactate and LPS assay before animals were killed at each time point.

**Rat models of burn**

Male Wistar rats weighing 190g - 250g were used. Animals were divided into two groups. In thermal group, they were subjected to a 30% total body surface area (TBSA) full-thickness scald burn injury (n=40). They were anesthetized with an intraperitoneal injection of 30g·L⁻¹ pentobarbital sodium (60mg·kg⁻¹) and then the dorsal hair was shaved. A 30% TBSA full-thickness burn was created on the back of the rats in boiling water at 98°C-100°C for 12s. Rats were resuscitated immediately after thermal injury with 50g·L⁻¹ glucose saline solution (50mL·kg⁻¹) intraperitoneally. In the control group (n=10), rats were exposed to the room-temperature water. Animals in thermal group were killed at 3, 6, 12 and 24h after burn. Blood samples were collected aseptically from cervical artery and portal vein before the rats were killed at each time point.

**Rat models of acute necrotizing pancreatitis (ANP)**

Male Wistar rats weighing 270g-330g were randomly divided into two groups. In the ANP group (n=27), animals were anesthetized with 30g·L⁻¹ pentobarbital sodium (60mg·kg⁻¹, ip). After medium laparotomy, the duodenum was mobilized and the pancreatic duct was identified at its duodenal junction. ANP was induced by a continuous inverse infusion of sodium taurocholate (50g·L⁻¹, 1mL·kg⁻¹) and trypsin (1.67×10⁵U·kg⁻¹) into the main pancreatic duct. Animals were immediately given saline (50mL·kg⁻¹) subcutaneously after injury. In control group (n=6), animals were treated identically with infusion saline. Blood samples were taken aseptically from cervical artery and portal vein at 2, 8, 24 and 48h after injury.

**D(-)-lactate determination**

The plasma from systemic blood samples was obtained and subjected to a deproteination and neutralization process by acid/base precipitation using perchloric acid and potassium hydroxide. The protein-free plasma was then assayed for D (-)-lactate concentration by enzymatic-spectrophotometric method with minor modification[25].

**Lipopolysaccharide (LPS) determination**

The plasma from portal vein blood was also obtained and subjected to a deproteination and neutralization process by acid/base precipitation using perchloric acid and potassium hydroxide. The LPS levels of portal vein blood were assayed by the chromogenic limulus amebocyte lysate (LAL) test with a kinetic modification according to the test kit procedure[26].

**Morphologic studies**

Tissue samples of intestines were taken for morphologic study. Biopsies were fixed in 100mL·L⁻¹ neutral buffered formalin, embedded in paraffin, microtome sectioned at 4μm-6μm thickness, and stained with hematoxylin and eosin. Sections were examined under light microscope.

**Statistical analysis**

Data were expressed as means ± SD. The statistical significance of mean values between groups was evaluated by the Student’s t test. The relationship between circulating systemic D (-)-lactate and portal vein LPS concentrations was determined by the calculation of Pearson correlation coefficient. P<0.05 was considered to be significant.

**RESULTS**

**Kinetics of D(-)-lactate and lipopolysaccharide concentrations in plasma after gut ischemia/reperfusion in rats**

One hour of gut ischemia alone induced a slight increase in systemic blood D (-)-lactate and portal vein blood LPS concentrations (Table 1). Either D (-)-lactate or LPS concentrations had a further significant increase at 0.5h-2h after gut reperfusion (P<0.05-0.01), and decreased to normal at 6h. Meanwhile, correlation analysis revealed a significant correlation between systemic blood D (-)-lactate levels and portal vein blood LPS concentrations (r = 0.719, P<0.05).

| Groups                  | Time(h) | No. | D(-)-lactate (mmol/L) | LPS(EU/L) |
|-------------------------|---------|-----|-----------------------|-----------|
| Sham-operated control   | 0.5     | 10  | 0.234±0.072           | 380±84    |
| Gut ischemia            | 1       | 10  | 0.260±0.086           | 407±41    |
|                         | 1.5     | 10  | 0.269±0.092           | 453±129   |
| Gut ischemia/reperfusion| 0.5     | 10  | 0.489±0.179           | 576±244   |
|                         | 1       | 10  | 0.37±0.179            | 61±278    |
|                         | 2       | 10  | 0.25±0.062            | 56±167    |
|                         | 6       | 10  | 0.23±0.044            | 335±73    |
|                         | 24      | 10  | 0.28±0.025            | 28±81     |

Compared with sham-operated control, respectively: *P<0.05; **P<0.01.

**Alterations in plasma D(-)-lactate and LPS levels in thermal rats**

Results presented in Table 2 indicated that there was a significant increase both in circulating blood D (-)-lactate and portal vein blood LPS concentrations at 6h after injury, and kept significantly increasing to the end of our observation period (72h, P<0.01). In addition, correlation analysis revealed that there was a strong positive correlation between plasma levels of D (-)-lactate and LPS after injury (r = 0.877, P<0.01).

| Groups            | Time(h) | No. | D(-)-lactate (mmol/L) | LPS (EU/L) |
|-------------------|---------|-----|-----------------------|------------|
| Control group     | 3       | 10  | 0.275±0.175           | 118±37     |
| Thermal group     | 6       | 10  | 0.517±0.162           | 347±111    |
|                   | 12      | 10  | 0.619±0.208           | 670±139    |
|                   | 24      | 10  | 0.638±0.198           | 396±57     |

Compared with control group, respectively: *P<0.01.
Changes in plasma D(-)-lactate and LPS levels in ANP rats

In rats subjected to ANP, the levels of D(-)-lactate in systemic blood and LPS in portal vein blood began to increase at 2h after ANP (P<0.01) (Table 3), and peaked at 24h after injury. Furthermore, a marked correlation was noted between the changes in contents of plasma D(-)-lactate and LPS (r=0.798, P<0.01).

Table 3 Alterations in systemic blood D(-)-lactate levels and portal blood LPS content in ANP rats (mean±SD)

| Groups          | Time(h) | No. | D(-)-lactate (mmol/L) | LPS (EU/L) |
|-----------------|---------|-----|-----------------------|------------|
| Control group   | 6       | 6   | 0.157±0.044           | 105±7      |
| ANP group       | 2       | 6   | 0.328±0.063           | 301±131    |
|                 | 8       | 7   | 0.507±0.157           | 449±164    |
|                 | 24      | 7   | 0.653±0.216           | 611±210    |
|                 | 48      | 7   | 0.448±0.112           | 422±136    |

Compared with control group, respectively: P<0.01.

Gut pathology

Mucosal edema, necrosis, and the loss of the epithelium in mucosa, as well as vascular dilution, congestion, edema and inflammatory cell infiltration in the lamina propria were observed in small intestinal biopsies in three groups. The intestinal injury paralleled with the changes of plasma D(-)-lactate levels.

DISCUSSION

The present study showed that the intestinal damage caused by gut ischemia caused a slight increase in plasma concentrations of D(-)-lactate in systemic blood and LPS in portal vein blood. After gut ischemia followed by reperfusion, the plasma levels of D(-)-lactate and LPS significantly elevated, but declined to normal rapidly at 6h after reperfusion[27-30]. The intestinal damage mediated by burn injury or ANP displayed a more severe damage than that in gut ischemia/reperfusion. A remarked increase of plasma D(-)-lactate and LPS concentrations occurred at 6h, and 3h after insult, respectively, and persisting to the end of our observation. Moreover, the elevation of plasma D(-)-lactate levels in systemic blood was associated with increased plasma LPS contents in portal vein blood, and histological examination also exhibited intestinal injury in those three rodent models.

D(-)-lactate is produced by some bacteria including Klebsiella, Escherichia coli, Lactobacillus species, and Bacteroides species. It is an indigenous products in gut[24]. Normally, serum levels of D(-)-lactate in mammals are quite low. During the event that an ischemia/reperfusion insults, the mucosa is injured and intestinal permeability is increased, leading to an efflux of bacteria and the products of their metabolism[30-33], including D(-)-lactate into the circulation. Otherwise, the gut ischemic insult leads to a loss in normal host defenses against bacterial overgrowth, resulting in increased numbers of bacteria within the lumen of the infected intestine[34-36]. This bacterial proliferation would be expected to cause an increased bacterial metabolism with increased production of D(-)-lactate. Mammals do not possess the enzyme system to rapidly metabolize D(-)-lactate, thus, it passes through the liver with unchanged way and enters the peripheral blood early in the disease process. Thus, D(-)-lactate accumulation in the systemic circulation can generally be considered as a result of bacterial over growth and increase in gut permeability induced by some gastrointestinal disorders. Therefore, D(-)-lactate levels could be used as a predictor of intestinal injury. In fact, the elevation of plasma D(-)-lactate levels has been used as the predictor of bacterial infection in patients with short-bowel syndrome[37]. In rat model of acute mesenteric ischemia, D(-)-lactate was significantly elevated after gut ischemia, and the histopathological evaluation scores of intestinal injury were remarkably correlated to the plasma D(-)-lactate levels[38,39]. Recently, in clinical study, it has also been demonstrated that patients with mesenteric ischemia at laparotomy had significantly elevated D(-)-lactate levels in systemic circulation as compared with patients operated on for an acute abdomen or normal abdomen[40].

In conclusion, our data in these rat models suggest that the changes in D(-)-lactate concentrations paralleled with LPS concentrations, and correlated similarly with the intestinal histopathological alterations as well. Therefore, plasma D(-)-lactate in systemic circulation measurement would be a useful marker to evaluate intestinal injury and endoxemia following severe injuries.

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Edited by Ma JY