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• ORIGINAL RESEARCH •

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

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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: ① gut ischemia/reperfusion obtained by one hour of superior mesenteric artery occlusion followed by reperfusion; ② severe burn injury created by 30% of total body surface area (TBSA) full-thickness scald burn; and ③ ANP 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

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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[1-7]. 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/and endotoxin from gut[8-12]. 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)[13-20]. Therefore, it is important to know the intestinal injuries following a variety of insults (shock, burn injury, sepsis, and some critically surgical illness)[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℃ - 24℃ 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 is chemia. 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℃-100℃ 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 pancretitis (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 (1.67×10^{5}U·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 sodium 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. Biospies 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).

Table 1 The plasma contents of D(-)-lactate and lipopolysac charide in rats after gut ischemia / reperfusion insults (mean±SD)

Groups	Time (h)	No.	D(-)-lactate (mmol/L)	LPS(EU/L)
Sham-operated control		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[b]	576±244[a]
	1	10	0.373±0.179[a]	611±278[a]
	2	10	0.253±0.062	562±167[a]
	6	10	0.237±0.044	335±73
	24	10	0.228±0.025	283±81

Compared with sham-operated control, respectively: [a]P<0.05; [b]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).

Table 2 Changes in systemic blood D(-)-lactate levels and portal blood LPS content in thermal rats (mean±SD)

Groups	Time(h)	No.	D(-)-lactate (mmol/L)	LPS (EU/L)
Control group		10	0.275±0.175	118±37
Thermal group	3	10	0.371±0.123	159±83
	6	10	0.517±0.162[a]	347±111[a]
	12	10	0.619±0.208[a]	670±139[a]
	24	10	0.638±0.198[a]	396±57[a]

Compared with control group, respectively: [a]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	0.157±0.044	105±7
ANP group	2	6	0.328±0.063[a]	301±131[a]
	8	7	0.507±0.157[a]	449±164[a]
	24	7	0.653±0.216[a]	611±210[a]
	48	7	0.448±0.112[a]	422±136[a]

Compared with control group, respectively:[a]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[27-29]. 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 *Klebsilla*, *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.

REFERENCES

1 Liu CH, Liu C, Liu P, Xu LM. Seropharmalogical effects of Fuzheng Huayu decoction on rat Ito cell morphology and functions in culture. *China Natl J New Gastroenterol*, 1997;3:263-265
2 Li ZL. Diagnosis and treatment of multiple organ disorder and failure induced by severe infections. *Shijie Huaren Xiaohua Zazhi*, 1999;7:1074-1076
3 Li Y, Li ZL. Influence of gastric distension on jejunal fluid absorption and transmural potential difference in rats. *Xin Xiaohuabingxue Zazhi*, 1997;5:684-686
4 Dong HL. Intestinal permeability test and its clinical significance. *Shijie Huaren Xiaohua Zazhi*, 2000;8:562-563
5 Luo H, Wang LF, Imoto T, Hiji Y. Inhibitory effect and mechanism of acarbose combined with gymnemic acid on maltose absorption in rat intestine. *World J Gastroenterol*, 2000;6(Suppl 3):84
6 Ruan CP, Wang YH, Wang LG, Wang YX. Changes of neurotensin and endotoxin in rats with intestinal ischemia. *China Natl J New Gastroenterol*, 1996;2:200-202
7 Qin RY, Zou SQ, Wu ZD, Qiu FZ. Influence of splanchnic vascular infusion on?the content of endotoxins in plasma and the translocation of intestinal bacteria in rats with acute hemorrhage necrosis pancreatitis. *World J Gastroenterol*, 2000;6:577-580
8 Swank GM, Edwin EA. Role of the multiple organ failure: Bacterial translocation and permeability changes. *World J Surg*, 1996;20:411-417
9 Langkamp-Henken B, Donovan TB, Pate LM, Kudsk KA. Increased intestinal permeability following blunt and penetrating trauma. *Crit Care Med*, 1995;23:660-664
10 Koike K, Moore FA, Moore EE, Poggetti RS, Tuder RM, Banerjee A. Endotoxin after gut ischemia/reperfusion cause irreversible lung injury. *J Surg Res*, 1992;52:656-662
11 Wang XJ, Luo XD, Luo Q, Yang ZC. Effects of sera from burn patients on human hepatocytic viscoelasticity. *World J Gastroenterol*, 1998;4:60
12 Ren JY, Ojeas H, Lightfoot SA, Harty RF. Effects of capsaicin on stress-induced duodenal injury. *World J Gastroenterol*, 1998; 4(Suppl 2):53
13 Baue AE, Durham R, Faist E. Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): Are we wining the battle? *Shock*, 1998;10:79-85
14 Ammori BJ, Leeder PC, King RF, Barclay GR, Martin IG, Larvin M, McMahon MJ. Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. *J Gastrointest Surg*, 1999;3:252-262
15 Berg JW, Deitch EA, Li M, Specian RD. Homorrhagic shock induced bacterial translocation from the gut. *J Trauma*, 1988;28: 896-906
16 Li JY, Sheng ZY, Lu Y, Yu Y, Hu S, Zhou BT. Severe trauma induced intestinal barrier function injury and protection. *Shijie Huaren Xiaohua Zazhi*, 2000;8:1093-1096

17 Yue MX. Management of digestive diseases complicated with multi-organ dysfunctional failure. *Huaren Xiaohua Zazhi*, 1998; 6:277-279
18 Zhang P, Yang WM, Shui WX, Du YG, Jin GY. Effect of Chinese herb mixture, shock decoction on bacterial translocation from the gut. *World J Gastroenterol*, 2000;6(Suppl 3):74
19 Wu CT, Huang XC, Li ZL. Increased intestinal permeability and intestinal bacterial transposition. *Shijie Huaren Xiaohua Zazhi*, 1999;7:605-606
20 Zhang QH, Ni QX, Cai D, Zhang YL, Jiang YF, Wu SQ, Xiang Y, Yin BB, Zhang N, Hou LD. Somatostatin and growth hormone protection on multiple organ injury in acute necrotizing pancreatitis. *Huaren Xiaohua Zazhi*, 1998;6(Suppl 7):185-188
21 Tu WF, Li JS, Zhu WM, Li ZD, Liu FN, Chen YM, Xu JG, Shao HF, Xiao GX, Li A. Influence of Glutamine and caecostomy/colonic irrigation on gut bacteria/endotoxin translocation in acute severe pancreatitis in pigs. *Shijie Huaren Xiaohua Zazhi*, 1999;7:135-138
22 Ci XL, Wang BE, Zhang SW, Zhang NN. Alterations of gastrointestinal motility and mucosal barrier in shock rat model induced by endotoxin plus TNF-α. *Shijie Huaren Xiaohua Zazhi*, 1999;7:510-512
23 Yao YM, Yu Y, Wu Y, Lu LR, Sheng ZY. Plasma D(-)-lactate as a new marker for diagnosis of acute intestinal injury following ischemia-reperfusion. *China Natl J New Gastroenterol*, 1997;3: 225-227
24 Smith SM, Eng HK, Buccini F. Use of D-lactic acid measurement in the diagnosis of bacterial infections. *J Infect Dis*, 1986; 154:658-664
25 Brandt RB, Siegel SA, Waters MG, Bloch MH. Spectrophotometric assay for D(-)-lactate in plasma. *Anal Biochem*, 1980; 102:39-46
26 Yao YM, Tian HM, Wang YP, Yu Y, Shi ZG. Microassay for quantification of endotoxin in blood with new PCA treatment using chromogenic limulus amebocyte lysate. *Shanghai Fenxi Zazhi*, 1993;8:31-33
27 Zhu L, Yang ZC, Li A, Cheng DC. Protective effect of early enteral feeding on postburn impairment of liver function and its mechanism in rats. *World J Gastroenterol*, 2000;6:79-83
28 Xu GH, Shi BJ, Liu HY. Analysis of prognostic factors in 178 patients with acute pancreatitis. *Xin Xiaohuabingxue Zazhi*, 1997;5:723-724
29 Xie CG, Wang XP. Endotoxin and pancreatic damage. *Shijie Huaren Xiaohua Zazhi*, 2000;8:1039-1041
30 Ruan CP, Wang YH, Wang LG. Bacterial translocation from the gastrointestinal tract in rats with intestinal ischemia. *Xin Xiaohuabingxue Zazhi*, 1996;4:304-305
31 Yu Y, Tian HM, Shi ZG, Yao YM, Wang YP, Lu LR, Yu Y, Chang GY, Ma NS, Sheng ZY. Relationship between endotoxemia and dysfunction of intestinal immuno-barrier after scald in rats. *Huaren Xiaohua Zazhi*, 1998;6:703-704
32 Zhang WZ, Han TQ, Tang YQ, Zhang SD. Rapid detection of sepsis complicating acute necrotizing pancreatitis using polymerase chain reaction. *World J Gastroenterol*, 2001;7:289-292
33 Horton JW, Walker PB. Oxygen radicals, lipid peroxidation, and permeability changes after intestinal ischemia and reperfusion. *J Appl Physiol*, 1993;74:1515-1520
34 Fu XB, Yang YH, Sun TZ, Gu XM, Jiang LX, Sun XQ, Sheng ZY. Effect of intestinal ischemia-reperfusion on expressions of endogenous basic fibroblast growth factor and transforming growth factor β in lung and its relation with lung repair. *World J Gastroenterol*, 2000;6:353-355
35 Fu XB. Growth factors in treatment of digestive system organ injuries. *Xin Xiaohuabingxue Zazhi*, 1997;5:663-664
36 Yang YH, Fu XB, Sun TZ, Jiang LX, Gu XM. bFGF and TGFβ expression in rat kidneys after ischemic/reperfusional gut injury and its relationship with tissue repair. *World J Gastroenterol*, 2000;6:147-149
37 Li YS, Li JS, Li N, Jiang ZW, Zhao YZ, Li NY, Liu FN. Evaluation of various solutions for small bowel graft preservation. *World J Gastroenterol*, 1998;4:140-143
38 Murray M, Barbose JJ, Cobb CF. Serum D(-)-lactate levels as a predictor of acute intestinal ischemia in a rat model. *J Surg Res*, 1993;54:507-509
39 Marcod MA, Vila J, Gratacos J, Brancos MS, Jimenez de Anta MT. Determination of D(-)-lactate concentration for rapid diagnosis of bacterial infection of body fluids. *Eue J Clin Microbiol Infect Dis*, 1991;10:966-969
40 Murray M, Gonze MD, Nowak LR, Cobb CF. Serum D(-)-lactate levels as an aid to diagnosis acute intestinal ischemia. *Am J Surg*, 1994;167:5575-5578

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