Clinical study on gut barrier dysfunction and bacterial translocation in patients after duodenopancreatectomy surgery*

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ABSTRACT Objective: To investigate the relationship between bacterial translocation and acute systemic inflammatory state (SIRS) in patients who underwent duodenopancreatectomy surgery. Methods: 40 patients who underwent selective duodenopancreatectomy operations were observed. Blood were collected before surgery and 1, 3, 5d after surgery to detect plasma D-lactate and extract DNA.PCR analysis was performed with β -Galactosidase gene of Eschenchia coli and 16SrRNA gene as target gene. The SIRS of all the patients were observed for 10 days. Results: All the PCR results before operation were negative, while there was positive in 13 patients (32.5%, 13/40) after duodenopancreatectomy surgery. The positive PCR rate in SIRS was 85.7% (12/14), which was remarkably higher than that without SISR (3.8%, 1/26) ($p<0.01$). 92.3% of the patient (12/13) with positive PCR result had SIRS while 7.4% patients (2/27) with negative PCR result did have SIRS ($p<0.01$). The plasma levels of D-lactate in patient with positive PCR result was significantly higher than those of the patients with negative PCR result ($p<0.01$). The plasma levels of D-lactate in patient with SIRS was significantly higher than those of patients without SIRS ($p<0.01$). Conclusion: Increased intestinal permeability had relationship with bacterial translocation and BT was closely related to SIRS after duodenopancreatectomy operations. The positive PCR result might be a useful early warning sign of postoperative SIRS.

Key words: gut barrier dysfunction; bacterial translocation; D-lactate; PCR; SIRS

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Introduction

It has long been recognized that major trauma, shock, or burn injury can lead to an acute systemic inflammatory state (SIRS) as well as the multiple organ dysfunction syndrome (MODS). Because of the high mortality rate associated with the development of MODS, for over two decades an intense effort has been devoted towards trying to unravel the underlying mechanisms of this complex syndrome. Although the gut has been implicated in the development of SIRS and MODS experimentally and clinically, its exact role in the pathogenesis of SIRS and MODS remains controversial[1]. However, based on recent experimental evidence, the gut barrier dysfunction and bacterial translocation (BT) plays a role in the development of SIRS and MODS[2].

SIRS in patients who undergo duodenopancreatectomy surgery are common and often lead to serious consequences, including systemic inflammatory response, multiple organ dysfunction syndrome (MODS), multiple system organ failure (MSOF). When the gut barrier dysfunction and bacterial translocation occur in these patients, the mortality is high. However, the conventional research methods for bacterial translocation and intestinal permeability are unfeasible or too complicated to be performed in clinical tria1. D-1actate is produced by bacteria indigenous to the gastrointestinal (GI)tract. Mammals do not have the required enzyme system to metabolize D-lactate. Therefore, the determination of plasma D-1actate leve1 is a usefu1 postoperative indicator of increased intestina1permeability and gut barrier dysfunction in patients undergoing intraperitoneal operation [3]. The polymerase chain reaction(PCR)analysis has higher sensitivity than blood and mesenteric 1ymphnodes (MLN)cultures for assessing BT from the intestine[4]. This study was to investigate the intestinal permeability and bacterial translocation by using plasma D-1actate measurement and PCR analysis in patients receiving duodenopancreatectomy surgery, and to investigate the relationship between BT and acute systemic inflammatory state (SIRS).

1 Materials and methods

1.1 Patients and Blood samples Collection

From December 2009 to July 2010, forty pancreatic cancer patients (18 women, 22 men ;mean age: 55.8 y) undergoing duodenopancreatectomy surgery at the Affiliated Hospital of Qingdao University Medical College were studied. Patients with severe systemic disease functional impairment of critical organs and severe complication were excluded from the study. An informed consent was obtained from all the patients, the study was approved by the local ethics committee. The trial was approved by the institutiona1 review board of our hospital.

Serial venous blood samples were taken with aseptic tech-

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nique 1 d before operation and 1, 3, and 5 d after surgery. Blood was collected in sterile Na2EDTA anticoagulant tubes and stored at 4℃ to extraction DNA. The remaining blood samples were centrifuged at 4℃ and 2000 r / min for 10 min and aliquoted into 1.5-mL Eppendorf tubes, and stored at-80℃ for the determination of D-1actate.

1.2 D—Lactic acid Measurement

Each sample that was previously diluted in 2 volumes of 7% perchloric acid was mixed and centrifuged at 1,500 x g for 20 min. D-Lactate dehydrogenase, glycine buffer (pH 9.2), and NAD+ were purchased from Sigma Chemical Co. St. Louis, Mo. and were added to all samples. Individual sample blanks contained the sample and water. Simultaneous samples with certain amounts of D-lactic acid (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) were also prepared to generate a standard curve. All tubes were incubated in a 35℃ water bath for 1 h, and the A340 was determined with a Gilford UV-Vis model 300N spectrophotometer (Gilford Systems, Oberlin, Ohio). The sample D-lactic acid concentrations were determined by interpolation of points generated from the standard curve.

1.3 The Detection of Microbial DNA in the Blood

Whole blood samples were processed in aliquots of 200 to 400 uL for DNA extraction. Blood was transferred from Na2 EDTA tubes to sterile 1.5-mL Eppendorf tubes, red cells were lysed, and total DNA was extracted according to the protocol described of manufacturer in the blood bacterial genome DNA extracting kit (Biospin Whole blood Genomic DNA Extraction).

Since E. coli was the most common translocated bacteria which was reported in various animal experiments and human studies. DNA from clinical isolates of E. coli and B. fragilis was extracted. The primer of BG-1, BG-4; 16S rRNA+, 16S rRNA- (E. coli DNA) were used in PCR reaction(Table 1).

Table 1 Oligonucleotide primers used to amplify bacterial DNA

Primer Designate	Sequence of + and - Primers(nucleotides)	Gene Target	Size of amplicon (bp)
BG-1 (+ strand)	5'CTT TGC CTG GTT TCC GGC ACC AGA A-3' (201-225)	β -Galactosidase	
BG-4 (- strand)	5'AAC CAC CGC ACG ATA GAG ATT CGG G-3' (963-939)	gene of Eschenchia	762
16SrRNA+ (+strand)	5'GGA CTA CCA GGG TAT CTA AT-3'(806-787)	coli	
16SrRNA- (- strand)	5'GGA CTA CCA GGG TAT CTA AT-3'(484-504)	DNA coding for 16S ribosomal RNA	768

1.4 SIRS Observation

Systemic inflammatory response syndrome(SIRS) of patients were observed for 10days after operation. SIRS is manifested by two or more of the following conditions: (1) temperature>38℃ or <36℃ ; (2) heart rate>90 beats / min (3)respirator rate>20 breaths / min or PaC02<32 mm Hg;(4)WBC>12,000 cel1s / mm3 or <4000 cells / mm3 or>l0% immature (band) forms.

1.5 Statistical Analysis

All statistical calculations were carried out by SPSS 13.0 statistical software. Data were expressed as ($\bar{X}\pm$ S). Statistical analysis was performed by using T-test, AN0VA or Chi-square test. The accepted level Of significance was P<0.05.

2 Result

2.1 The result of PCR and SIRS

All the PCR result before operation were negative, while it was 13 positive patients (32.5%,13/40) after duodenopancreatectomy surgery (table 2). The PCR positive rate was 17.5% (7/40) at 1d, 22.5%(9/40) at 3d and 30%(12/40) after surgery respectively. There were no statistical significances among them (p >0.05). Among the positive PCR results, E. coli DNA was found in 69.2% (9/13), and 16SrRNA was obversed in all of the blood samples with E. coli DNA positive.

There were 14 patients with SIRS and 26 patients without SIRS after duodenopancreatectomy surgery. Among 14 patients with SIRS, 12 patients PCR result were positive. All of the 13 postive PCR patients, 12 patients were with SIRS. The Positive PCR rate in SIRS was 85.7% (12/14), which was remarkably higher than that of patients without SISR (3.8%, 1/26) (p<0.01). About 92.3% of the patient (12/13) with positive PCR result had SIRS while 7.4%of the patients (2/27) with negative PCR result did have SIRS(p<0.01). (Table2)

Table 2 The PCR result of blood sample from 4o patients undergoing duodenopancreatectomy surgery

Groups	n	PCR(+)	PCR analysis							
			BG				16SrRNA			
			0d	1d	3d	5d	0d	1d	3d	5d
SIRS	14	12	0	5	7	10	0	7	9	11
Non-SIRS	26	1	0	1	0	0	1	0	0	0

2.2 D−lactate, PCR and SIRS

The plasma levels of D-lactate increased in all patients undergoing duodenopancreatectomy surgery. The plasma levels of D-lactate in patient with positive PCR result was significantly higher than those of the patients with negative PCR result ($p<0.01$). The plasma levels of D-lactate in patient with SIRS was significantly higher than those of the patients without SIRS ($p<0.01$). (Table 3).

Table 3 The plasma levels of D-lactate (ug/ml) undergoing duodenopancreatectomy surgery

Groups	n	0d	1d	3d	5d
PCR(+)	13	0.42± 0.17	15.45± 3.34*	13.91± 1.93*	11.12± 2.10*
PCR(-)	27	0.43± 0.21	4.86± 1.71	4.07± 2.31	3.26± 2.11
SIRS	14	0.42± 0.19	15.23± 3.30**	13.0± 1.93**	11.25± 2.07**
Non-SIRS	26	0.43± 0.20	4.57± 0.81	3.72± 1..48	3.12± 1.44

*P<0.01 PCR(+) group siginificantly higher than PCR(-) group;

**P<0.01 SIRS group siginificantly higher than Non-SIRS group

3 Discussion

Bacterial translocation (BT) is a phenomenon in which live bacteria or the products cross the intestinal barrier. Bacterial translocation is defined as the passage of viable indigenous bacteria from the gastrointestinal tract to extraintestinal sites, such as the mesenteric-lymph-node complex, liver, spleen and bloodstream. The bacterial translocation may result in the ingress of viable bacteria and their antigens with the development of sepsis, initiation of a cytokine mediated systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), and death[5-6]. It is generally accepted that experimental animal, especially with severe trauma and / or hemorrhagic shock, can develop bacteria1 translocation, but if bacterial translocation takes place in clinical patients is stil1 controversial[7].

The conventional blood culture techniques frequently failed to demonstrate bacterial invasion to bloodstream. PCR analysis makes it possible to detect bacterial DNA in bloodstream and provides a direct evidence of bacterial translocation. It can be used to confirm presence of bacteria in bloodstream and it is fast and sensitive, which is essential for clinical application [8]. D-lactate is a product of bacterial fermentation and is produced by many of the bacteria found in the human gastrointestinal (GI) tract. The increase in D-lactate might thus reflect an efflux of bacteria and/or its products into circulation because of mucosal intestinal injury. As mesenteric ischemia first causes mucosal injury and then bacterial proliferation, normally low serum levels of D-lactate increase as the products of bacterial metabolism enter the circulation [3]. Mammals do not have the enzyme system required to metabolize D-lactate. Therefore, it is passed through the liver and enters the peripheral circulation early in the disease process. The determination of plasma D-1actate leve1 is a usefu1 postoperative indicator of increased intestina1permeability and gut barrier dysfunction in patients undergoing intraperitoneal operation[9].

In the study, the plasma levels of D-lactate in patient with positive PCR result was significantly higher than those of the patients with negative PCR result ($p<0.01$). The plasma levels of D-lactate in patient with SIRS was significantly higher than those of the patients without SIRS ($p<0.01$). The results suggested that increased intestina1 permeability and gut barrier dysfunction occurred in patients undergoing duodenopancreatectomy operations and were followed by bacterial translocation resulting in SIRS. The possibility of suffering SIRS was increased significantly in positive PCR patients than that in negative PCR patients. The positive PCR rate in SIRS was 85.7% (12/14), which was remarkably higher than that patients without SISR (3.8%, 1/26) ($p<0.01$). About 92.3% the patient (12/13) with positive PCR result had SIRS while 3.7% the patients (1/27) with negative PCR result did ($p<0.01$). The study revealed that BT was closely related to SIRS after duodenopancreatectomy operations, the positive PCR result might be a useful early warning sign of postoperative SIRS.

In this study, it revealed that BT may occur after abdominal surgery and the severity of trauma and intestinal ischemia might be one of the significant factors for BT. This pathophysio1ogical phenomenon is attributable to visceral angiospasm induced by trauma and anesthesia, intestinal ischemia and anoxemia caused by blood loss, the production of vasoactive factors and prostaglandins, ischemia-reperfusion injury[10] and the release of inflammatory cytokines[11] during abdominal surgery. The findings of this study suggested that increased intestina1 permeability could 1ead to bacterial translocation in patients undergoing duodenopancreatectomy. BT was closely related to SIRS after duodenopancreatectomy operations, the positive PCR result might be a useful early warning sign of postoperative SIRS. The improvement in surgical techniques, shortening of operation time, reduction in trauma and bloodloss might be important in lessening BT.

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胰十二指肠切除术后肠黏膜屏障损伤与肠道细菌移位的临床研究*

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摘要 **目的**:探讨胰十二指肠切除手术后肠道细菌移位(BT)与术后全身炎症反应综合征(SIRS)关系。**方法**:40 例择期行胰十二指肠切除手术患者,于术前和术后 1、3、5 天采集外周血,进行血浆 D-乳酸,全血细菌 DNA 检测. 全血 DNA 提取后进行 PCR 扩增,采用靶基因为大肠杆菌特异性 β 半乳糖苷酶基因和 16SrRNA 基因。观察患者术后 10 天以监测 SIRS 情况。**结果**:术前 PCR 检测全血细菌 DNA 均为阴性,术后共有 13 例阳性。术后出现全身炎症反应综合征(SIRS)的患者 PCR 阳性率为 85.7 %(12/14),无 SIRS 组为 3.8%(1/26) (P< 0.01) 。PCR 阳性组 SIRS 发生率为 93.2%(12/13),阴性组为 7.4%(2/27)(P <0.01).PCR 阳性的患者外周血血浆 D-乳酸浓度较 PCR 阴性者明显升高(P< 0.01) ,有 SIRS 的患者外周血血浆 D-乳酸浓度较无 SIRS 患者明显升高(P< 0.01)。**结论**:胰十二指肠切除术后肠黏膜屏障损伤与 BT 关系密切,术后 SIRS 和与 BT 密切相关。PCR 技术对术后 SIRS 有较好的早期预警价值。

关键词:肠黏膜屏障损伤;细菌移位;D-乳酸;PCR;全身炎症反应综合征

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