# Changes in Endotoxin- and Platelet-activating Factor of Rats at Different Periods after Acute Small-bowel Obstruction Occurrence

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**Abstract: Objective:** This study aimed to investigate the levels of endotoxin and platelet-activating factor (PAF) in plasma and tissue homogenates during different phases of acute small-intestine obstruction of rats. **Methods:** Seventy-two specific pathogen-free Wistar rats were completely and randomly divided into the control, sham-operated, and acute small-intestine obstruction groups. Operation was performed on rats in each group after intraperitoneal administration of anesthesia. After the operation, venous blood was phlebotomized at four different time points for examination, and part of the ileum was taken for the tissue homogenate. The levels of endotoxin and PAF in venous blood and tissue homogenate were examined by ELISA. **Results:** The levels of plasma endotoxin increased evidently after operation, achieved its peak value after 24 h, and then decreased, showing a double-peak curve. The levels of PAF in both plasma and tissue homogenates as well as the endotoxin in the tissue homogenate increased evidently after operation compared with that of the normal control group ($P < 0.01$). The level of endotoxin had a positive correlation with PAF ($r = 0.656$, $P < 0.01$). **Conclusions:** The levels of plasma endotoxin can be used to evaluate the function of the intestinal mucosal barrier, and as a sensitive cytokine, PAF can determine disease progression and offer evidence for early intervention.
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## 1. Introduction

An endotoxin is a lipopolysaccharide component in the cell wall of gram-negative bacteria and is the main trigger factor of inflammatory reaction. Under normal physiological status, a large amount of endotoxins exist in the intestinal tract. Given that mucosal barrier function is complete, bacteria and endotoxins in the intestinal tract have difficulty invading the human body (Swank and Deitch, 1996). Platelet-activating factor (PAF) is a kind of endogenous phospholipid medium with wide biological activities. PAF can be generated by mastocytes, endothelial cells, macrophages, and polymorphonuclear leukocytes. In the human body, the combination of PAF with a specific PAF receptor can promote blood platelet and neutrophil accumulation as well as cell degranulation and release of oxygen free radicals (OFR), tumor necrosis factor (TNF), interleukin (IL), arachidonic acid, and metabolic products, thus increasing vasopermeability, promoting thrombosis, and inducing smooth muscle contraction. Furthermore, PAF serves an important function in the occurrence and development process of a series of related diseases (Anderson et al., 1991) such as inflammation, microcirculatory disturbance, and gastrointestinal mucosal injury (Prescott et al., 2000).

Intestinal barrier is a kind of complex defense system composed of the intestinal mucosal epithelium, rete malpighii, mucosal immune system, normal intestinal microflora, intestinal endocrine function, normal intestinal peristalsis, and other links (Magnotti and Deitch, 2005). This barrier can effectively prevent pathogenic microorganisms from invading the body (Deitch, 2002). However, under the conditions of operation, trauma, infection, ileus, malnutrition, jejunitas, and chemotherapy, intestinal barrier injuries can occur (Gosain and Gamelli, 2005), thus enabling endotoxins to invade the blood and causing enterogenous endotoxemia (Morencos et al., 1995) and multiple organ dysfunction syndrome (Han, 2002; Steinberg, 2003). After ileus occurs, microcirculatory disturbance of intestinal wall tissues, nutritional disturbance, infection, excessive release of inflammatory mediators, cell apoptosis, and other multiple routes can all result in intestinal barrier function injury (Sobhani et al., 1992). Both intestinal flora disturbance and intestinal mucosal permeability increase can cause intestinal bacteria and endotoxin to invade the blood. Therefore, endotoxins can sensitively reflect intestinal mucosal barrier function and can serve as an objective indicator of the extent of intestinal mucosal barrier injury and can assess enterogenic

infection and disease prognosis for ileus disease (Smith et al., 1986). As the central organ of stress reaction and the initiation organ of multiple organ failure (Marshall et al., 1995; Lan and Tang, 2003; Leaphart and Tepas, 2007), the intestinal tract increasingly becomes a concern. Translocation of bacteria and severity extent of endotoxemia are closely related to the extent of intestinal barrier injury and directly influence the prognosis of patients (Higashi et al., 2003). Therefore, the prevention and treatment of intestinal barrier function injury is of great significance (Mannon, 2009).

As a kind of endogenous phospholipid medium with wide biological activities, PAF serves an important function in the occurrence and development process of gastrointestinal mucosal injury (Wang et al., 1999). In China, relevant research on PAF level in the ileus remains lacking. This study used the intestinal obstruction rat model to detect concentrations of endotoxin and PAF in blood plasma and small-intestine tissue homogenates at different time points after the occurrence of acute ileus to understand the rules of endotoxin and PAF change after such occurrence as well as to assess the disease condition of ileus to provide a theoretical basis for early clinical intervention.

## 2. Materials and methods

Animals and Grouping: Seventy-two specific pathogen-free Wistar male rats with body weight of 250 g to 300 g were provided by the Qingdao Experimental Animal and Animal Experiment Center. Before the experiment, jejunitas was performed for 12 h, but the rats could freely drink. These rats were randomly divided into the following three groups: group A (the normal control group, $n = 8$), group B (the sham-operated group, $n = 32$), and group C (the acute ileus group, $n = 32$). This study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee of Qingdao University

Model Preparation: The rats were anesthetized with 40 mg/kg of 2% amobarbital sodium by intraperitoneal injection. After the administration of anesthesia, the rats were fixed. Routine skin disinfection was performed for the operation field, and operations were conducted under aseptic conditions. Median abdominal skin was cut open to the abdominal cavity layer by layer to form an incision of approximately 3.5 cm, and subsequent operations were performed according to the following procedure: For the rats in group A, specimens were acquired after anesthesia. For the rats in group B, only intestines were stirred after opening the abdominal cavity. For group C, small intestines were ligatured with one piece of (4 m) silk suture 5 cm away from the terminal ileum. For groups B and C, the abdominal cavity was closed, and nutritional support was administered.

Specimen Collection: For the rats in group A, peripheral venous blood was collected after injecting anesthesia, and the rats were killed by cutting the neck. For the remaining groups, the rats were again anesthetized at 8, 24, 72, and 168 h after model preparation (four batches, eight rats/batch), and venous blood was collected. After the upper intestinal tissues of the upper ileus were taken, various groups of rats were killed by cutting the neck. The venous blood was centrifuged at a low temperature of 4 °C at 3000 rpm for 30 min to obtain the supernatant. Subsequently, 300 μL of supernatant was placed into an aseptic EP tube and stored at −70 °C for detection. After mesenteries were removed from the intestinal wall tissue, the tissue was washed with ice-cold normal saline, prepared in a high-speed tissue homogenizer within the super-clean bench at 15000 g into 20% of tissue homogenate, and then centrifuged for 10 min at a low temperature of 4 °C at 3000 g. The supernatant was placed into an aseptic plastic EP tube and stored at −70 ℃ for detection.

Specimen Detection: PAF and endotoxin kits (American R&D Systems Company) were balanced to room temperature (18 ℃ to 25 ℃), and then the ELISA plate was removed. Detections were performed in strict accordance with the operation steps of the kit instructions. At a wavelength of 450 nm, optical density (OD) values of endotoxin and PAF in various walls were read on the enzyme-labeled instrument. With the standard concentration as abscissa and the corresponding OD value as ordinate, the linear regression curve of standard substance was drawn. According to the curve equation, concentration values of various specimens were calculated.

Statistical Analysis: All obtained data were expressed as mean ± standard deviation ($\bar{x} \pm s$), and the SPSS 17.0 statistical software package was used for statistical analysis. In addition, *t* test was used for comparison between two groups, ANOVA was used for comparisons among intragroup multiple mean values, and linear correlation analysis was used for the correlation analysis of two variables. $P < 0.05$ was considered as statistically significant.

## 3. Results

Analysis of Changes in Endotoxin and PAF Concentrations: All experimental animals survived in the experimental period. At 8 h after ileus, endotoxin in rat blood plasma significantly increased ($P < 0.01$), reached the peak, then reduced slightly, and increased again, presenting a double-peak change. At various time points, the acute ileus group has significantly

more endotoxin in the blood plasma than the normal control group ($P < 0.05$). In addition, PAF in blood plasma and endotoxin and PAF in tissue homogenate at 8 h after ileus significantly increased ($P < 0.01$) and then reached the peak at 168 h. At various time points, endotoxin and PAF concentrations in the blood plasma and tissue homogenates of the acute ileus group were all higher than those of the sham-operated group (see Table 1).

**Table 1.** Comparison of endotoxin and plasma PAF level in the plasma and normal colon wall after the rat model was established among the three groups ($\bar{x} \pm s$)

| Sample | Parameter | Group | N | 8 h | 24 h | 72 h | 168 h |
|---|---|---|---|---|---|---|---|
| Plasma | PAF | A | 8 | 458.38±31.49 | 458.38±31.49 | 458.38±31.49 | 458.38±31.49 |
| | (pg/ml) | B | 32 | 692.88±53.32** | 868.50±40.27** | 1297.63±39.89** | 944.75±41.63** |
| | | C | 32 | 722.63±36.59** | 915.13±38.74** # | 1433.88±48.84**## | 1444.75±41.63**## |
| | Endotoxin | A | 8 | 26.32±1.37 | 26.32±1.37 | 26.32±1.37 | 26.32±1.37 |
| | (EU/ml) | B | 32 | 32.55±2.19** | 42.87±3.34** | 40.00±4.45** | 32.83±2.05** |
| | | C | 32 | 33.18±2.24** | 43.06±3.65** | 40.57±3.26** | 48.72±1.86** ## |
| Tissue | PAF | A | 8 | 3029.38±171.58 | 3029.38±171.58 | 3029.38±171.58 | 3029.38±171.58 |
| | (pg/ml) | B | 32 | 4048.75±198.13** | 5299.38±304.93** | 7934.75±367.17** | 5853.13±263.79** |
| | | C | 32 | 4620.63±205.47** | ##5500.00±261.48** | 8144.38±351.85** | 8362.50±313.56**## |
| | Endotoxin | A | 8 | 87.55±5.89 | 87.55±5.89 | 87.55±5.89 | 87.55±5.89 |
| | (EU/ml) | B | 32 | 156.73±17.36** | 187.89±7.29** | 198.09±10.35** | 161.96±12.40** |
| | | C | 32 | 156.36±15.49** | 188.82±9.53** | 208.95±16.32** | 219.27±15.71** ## |

Note: Groups B and C versus group A, *$P$< 0.05, **$P$ < 0.01; Group B versus group C, #$P$ < 0.05, ##$P$ < 0.01.

## 4. Discussion

This study investigated the rule of change in endotoxin and PAF at different periods by detecting endotoxin and PAF concentrations in blood plasma and small-intestine tissue homogenates at different time points after acute small-bowel obstruction of rats to provide a clinical judgment basis for the progress of ileus disease condition and to provide a theoretical basis for early intervention. After abdominal operation, endotoxin in the blood plasma was significantly higher in the normal control group ($P < 0.01$). At 24 h, the endotoxin content of the acute ileus group reached the peak. After 72 h, the endotoxin content slightly reduced and then continuously increased. At 168 h, the endotoxin content again reached the peak. Furthermore, at any period, the endotoxin content of the acute ileus group was greater than that of the sham-operated group. This phenomenon was related to surgical stress. Qiao et al. (2004) believed that both anesthesia and operation trauma stimulus during abdominal surgical operation could cause visceral vascular spasm. In addition, intraoperative hemorrhage and intestinal ischemia and hypoxia status caused intestinal mucosal injury and intestinal barrier function damage. Therefore, endotoxemia and bacterial translocation occurred. In this experiment, at postoperative 8 h, the endotoxin content of the sham-operated group was significantly higher than that of the normal group ($P < 0.01$), reaching the peak at postoperative 24 h and then continuously decreasing, which also confirmed this viewpoint. After postoperative fasting, early ileus symptoms were not apparent, and operative stress reaction interfered. Thus, no significant difference in the endotoxin content of blood plasma was found between the acute ileus group and the sham-operated group. After postoperative 72 h, the influence of acute stress on the body gradually reduced, and with the increase of ileus time, intestinal barrier function injury gradually aggravated. In addition, the influence of intestinal barrier injury on endotoxin translocation would become the predominant factor. Therefore, endotoxin contents in blood plasma and tissue homogenate significantly increased and reached the peak at 168 h after ileus, which is significantly higher in the sham-operated group ($P < 0.01$). Endotoxin content in blood plasma is evidently associated with the extent of intestinal mucosal injury and can thus be used as a sensitive indicator for evaluating the extent of intestinal barrier function injury.

In the early stage after abdominal operation, PAF contents in blood plasma and tissue homogenates were significantly higher in the normal control group ($P < 0.01$), but no significant difference was found between the sham-operated group and the acute ileus group, which was also possibly associated with operative stress. At postoperative 8 h, PAF content in the tissue homogenates of the acute ileus group was significantly higher than that of the sham-operated group ($P < 0.05$), which was possibly associated with intraoperative intestinal ligation. At 24 h after ileus, PAF content in the blood plasma was significantly higher in the sham-operated group ($P < 0.05$), but no difference was found for PAF content in the tissue homogenates, suggesting that the biological activity of PAF was not only limited to local ileus, but is also more importantly involved in systemic inflammatory reaction in the cycle. At 72 h after ileus, PAF content in blood plasma was significantly higher in the sham-operated group ($P < 0.01$), whereas endotoxin content slightly reduced and could not illustrate the

inflammatory development trend. PAF can therefore be used as a sensitive inflammatory factor for evaluating the systemic inflammatory development trend. In the experimental process, the life quality of the experimental animals at postoperative 72 h significantly reduced, and spirits were low. In addition, response and resistance capabilities to external stimuli evidently reduced. The mild endotoxemia after operation trauma causes systemic inflammation, thus stimulating the release of inflammatory factors such PAF. With the increase of ileus time, intestinal barrier injury is aggravated, endotoxin further invades the blood, and PAF release increases, which cause the excessive activation of cytokines such as OFR, prostaglandin, IL, and TNF and activate the "cascading waterfall effect" of inflammatory factor that will cause a "second strike" to the body (Deitch, 2002), thus resulting in systemic inflammatory response syndrome. Recent studies suggested that PAF receptor antagonist or PAF acetylhydrolase could relieve the inflammatory reaction of shrimp alkaline phosphatase (SAP), improve pancreatic microcirculatory disturbance, and evidently improve SAP prognosis (Lane et al., 2001). Some studies reported that the application of PAF receptor antagonist could apparently improve gastric mucosal ischemia caused by endotoxin shock and relieve the morphological damage to the stomach and duodenum caused by OFR in hemorrhagic shock (Debek et al., 1998). Thus, as found in this study, PAF is not only involved in the inflammatory reaction process of ileus but is also more sensitive to the inflammatory reaction development trend than endotoxin, which provides an experimental basis for monitoring the systemic inflammatory reaction of ileus, early application of PAF receptor antagonist or acetylhydrolase intervention, interrupting the "cascading waterfall effect" of inflammatory factor, and improving ileus prognosis. Moreover, the results provide a reference indicator for preparing the quantified standard of ileus treatment opportunity selection.

**5.References**


[1] Anderson BO, Bensard DD, Harken AH. The role of platelet activating factor and its antagonists in shock, sepsis and multiple organ failure. Surg Gynecol Obstec 1991; 172(5):415-424.

[2] Debek W, Chyczewski L, Makarewicz M. Platelet-activating factor receptor-antagonist (BN 52021) stabilizes the oxidative-antioxidative balance and attenuates the morphological changes in the gastrointestinal tract in experimental hemorrhagic shock. Exp Toxicol Pathol 1998; 50(1):19-25.

[3] Deitch EA. Bacterial translocation or lymphatic drainage of toxic products from the gut: what is important in human beings? Surgery 2002; 131(3):241-4.

[4] Gosain A, Gamelli RL. Role of the gastrointestinal tract in burn sepsis. J Burn Care Retmbil 2005; 26(1):85-91.

[5] Han DW. Intestinal endotoxemia as a pathogenetic mechanism in liver failure. World J Gastroenterol 2002; 8(6):961-5.

[6] Higashi H, Shida H, Ban K, Yamagata S, Masuda K, Imanari T, Yamamoto T. Factors affecting successful palliative surgery for malignant bowel obstruction due to peritoneal dissemination from colorectal cancer. Jpn J Clin Oncol 2003; 33(7):357-9.

[7] Lan C, Tang CW. Effects of substance P on the activity of intestinal mucosal mast cells in rats with multiple organ failure. Chin J Dig 2003;23(52):271-4.

[8] Lane JS, Todd KE, Gloor B, Chandler CF, Kau AW, Ashley SW, Reber HA, McFadden DW. Platelet activating factor antagonism reduces the systemic inflammatory response in a murine model of acute pancreatitis. J Surg Res 2001; 99(2):365-70.

[9] Leaphart CL, Tepas JJ 3rd. The gut is a motor of organ system dysfunction. Surgery 2007; 141(5):563-569.

[10] Magnotti LJ, Deitch EA. Mechanisms and Significance of Gut Barrier Function and Failure. In: Rolandelli RH, Bankhead R, Boullata JI, Compher CW, editors. Clinical Nutrition: Enteral and Tube Feeding. 4 Ed. Phil-adelphis: Elsevler. 2005:23-31.

[11] Mannon P. Gut permeability and colitis. Gastroenterology 2009; 137(2):732-4.

[12] Marshall JC, Cook DJ, Chiston NV, Bernard GR, Sprung CL, Sibbald WJ. Multiple organ dysfunction score: A reliable descriptor of a complex clinical outcome. Crit Care Med 1995; 23(10):1638-52.

[13] Morencos FC, de las Heras Castaño G, Martín Ramos L, López Arias MJ, Ledesma F, Pons Romero F. Small bowel bacterial overgrowth in patient with alcoholic cirrhosis. Dig Dis Sci 1995; 40(6):1252-6.

[14] Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. Annu Rew Biochem 2000; 69:419-445.

[15] Qiao Z, Li ZL, Li JY, Lu LR, Lu Y, Yu Y, Li JY, Sun D. Clinical study on bacterial translocation and gut barrier dysfunction in postoperative patients. Chin J Emerg Med 2004; 13(1):466-8.

[16] Smith SM, Eng RH, Buccini F. Use of D-lactic acid measurements in the diagnosis of bacterial infections. Infect Dis 1986; 154(4):658-64.

[17] Sobhani I, Denizot Y, Vissuzaine C, Vatier J, Benveniste J, Lewin MJ, Mignon M. Significance and regulation of gastric secretion of platelet-activating factor (PAF-acether) in man. Dig Dis Sci 1992; 37(10):1583-92.
[18] Steinberg SM. Bacterial translocation: what it is and what it is not. Am J Surg 2003; 186(3):301-5.
[19] Swank GM, Deitch EA. Role of the gut in multiple organ failure: bacterial translocation and Permeability changes. World J Surg 1996; 20(4): 411-7.
[20] Wang X, Sun Z, Börjesson A, Andersson R. Inhibition of platelet-activating factor, intercellular adhesion molecule 1 and platelet endothelial cell adhesion molecule 1 reduces experimental pancreatitis-associated gut endothelial barrier dysfunction. Br J Surg 1999; 86(3):411-6.

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