

# Pathogenetic effects of platelet activating factor on enterogenic endotoxemia after burn

Pei Wu Yu<sup>1</sup>, Guang Xia Xiao<sup>2</sup>, Wei Ling Fu<sup>2</sup>, Jian Cheng Yuan<sup>2</sup>, Li Xin Zhou<sup>2</sup> and Xiao Jian Qin<sup>2</sup>

**Subject headings** Platelet activating factor; burn; endotoxemia ; intestinal permeability

Yu PW, Xiao GX, Fu WL, Yuan JC, Zhou LX, Qin XJ. Pathogenetic effects of platelet activating factor on enterogenic endotoxemia after burn. *World J Gastroentero*, 2000;6(3):451-453

## INTRODUCTION

Previous clinical and experimental studies have indicated that an early endotoxemia occurred after a major burn. It is unlikely that burn wound sepsis is the source of circulating endotoxin in less than 12 hour after burn. Increasing evidence demonstrates that the bacteria and endotoxin in the gastrointestinal tract can pass through the gut barrier into blood circulation to form enterogenic endotoxemia following burn<sup>[1-3]</sup>. However, its pathogenesis remains poorly understood. Platelet activating factor (PAF), an endogenous phospholipid mediator, has recently been proposed as a critical mediator in shock, sepsis and multiple organ failure<sup>[4,5]</sup>. In this study, the relationship between changes of PAF and enterogenic endotoxemia was observed on rat models with 30% TBSA III° burn. The purpose was to investigate the pathogenetic effects of PAF on the occurrence of enterogenic endotoxemia after burn.

## MATERIALS AND METHODS

### Animals

Wistar rats, male or female, weighing 220 g ± 30 g were used, they were provided by Laboratory of Animal Experiment, Institute of Burn Research, Third Military Medical University.

### Experimental design

Animals were randomly divided into three groups. Group 1 (*n* = 10): normal rats served as control. Group 2 (*n* = 40): burned rats that had undergone

30% TBSA III° burn. Group 3 (*n* = 40): treated rats that received PAF antagonist WEB 2170 (5 mg/kg) by intraperitoneal injection after burn. WEB 2170 was provided by Boeringer Ingelheim *Pharmac*-euticals Inc, Germany.

Animals were killed on 6, 12, 24 and 48 hours postburn. Blood and terminal ileum were obtained from all animals for assay of PAF and endotoxin.

### Burn model

Rats were anesthetized intraperitoneally with ketamine hydrochloride 80 mL/kg body weight, and their backs were shaved. They were placed in a mould that left approximately 30% area of their body surface exposed. These exposed surface s were immersed in 92°C water for 18s. This type of burn injury is a full-thick ness burn. Animals were resuscitated with 40 mL/kg of lactated Ringer's solution.

### Measurement of PAF contents in blood and intestinal tissue

Blood (1mL) was collected into polypropylene tube containing 5 mL of methanol. The methanolic extract was separated by centrifugation. The supernata nts were collected and chloroform and water were added to effect phase separation. The lower chloroform-rich phase contained all PAF activity. Chloroform was evaporated under a stream of nitrogen. The samples were stored under -20°C. Segments of ileum tissue (200 mg) were added to 2 mL of 0.25% bovine serum albumin, and after homogenization, the mixture was added to 2 mL of cold acetone, and then centrifuged. Two mL of chloroform as added to the superna tants. After a further centrifugation, the thin layer chloroform containing PAF was collected and evaporated by nitrogen. PAF activity was bioassayed by the aggregation of rabbit washed platelets.

### Measurement of intestinal mucosal permeability

After overnight fast, rats were anesthetized, a midline abdominal incision was made. Fifteen cm segments of ileum were isolated, cannulated proximally and distally, and perfused continuously with saline at rate of 1 mL/min-2 mL/min. <sup>99m</sup>Tc DTPA (5.55-7.4MBq/kg) was injected via the carotid ve in and allowed to equilibrate for 30 min. After that, a 10 min perfusion fluid and 1mL blood were collected for measurement of activity of <sup>99m</sup>Tc

<sup>1</sup>Department of General Surgery, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

<sup>2</sup>Institute of Burn Research, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

Dr. Pei Wu Yu, graduated from the Third Military Medical University in 1984, now associate professor of surgery, majoring in gastrointestinal surgery, having 28 papers published.

Project supported by the National Natural Science Foundation of China, No.39290700.

Correspondence to: Dr. Pei Wu Yu, Department of General Surgery, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

Tel. 0086-23-68754097

Received 2000-01-11 Accepted 2000-02-16

DTPA. The animals were killed and the perfused ileal segment was excised and weighed. DTPA clearance was calculated using the following formula:  $DTPA \text{ clearance} = (\text{cpm perfusate} \times Q) / (\text{cpm plasma} \times W)$ , and was expressed as  $\text{mL/min} \cdot 100\text{g}$ . Where Q is the rate of perfusion; W, the weight of perfused ileum.

#### Measurement of plasma endotoxin

Plasma endotoxin was assayed with chromogenic limulus amoebocyte lysate technique.

#### Statistical analysis

All data were expressed as  $\bar{x} \pm s$ , and statistical analyses were made using Student's *t* test.

### RESULTS

#### The changes of PAF content

PAF contents of blood and intestinal tissue in burn group were significantly higher than those in control group ( $P < 0.01$ ). The peak level occurred at 12 h postburn. In PAF-antagonist treatment group, the PAF contents of blood and intestinal tissue were significantly decreased compared with burn group, but were higher than those in control group

(Table 1).

#### The changes of intestinal mucosal permeability

The intestinal mucosal permeability increased significantly at 6h postburn and kept increasing during 48 h postburn compared with control group. The intestinal mucosal permeability in treatment group was lower than those in burn group (Table 2).

#### The changes of plasma endotoxin

The levels of plasma endotoxin in burn group were significantly higher than those in control group. The levels of plasma endotoxin in treatment group were significantly lower than those in burn group (Table 3).

#### Correlation analysis

The correlations between intestinal PAF and intestinal mucosal permeability, blood PAF and plasma endotoxin, intestinal mucosal permeability and plasma endotoxin in burn group were analyzed. The results showed positive correlation among the above three pairs with  $P < 0.01$ ,  $r = 0.94$ ,  $0.93$  and  $0.95$  respectively.

**Table 1 Levels of PAF in blood and intestinal tissue ( $\bar{x} \pm s$ )**

Group	n		After burn (h)			
			6	12	24	48
Control	10					
Blood( $\mu\text{g/L}$ )		$0.56 \pm 0.07$				
Intestine( $\text{ng/g}$ )		$0.41 \pm 0.06$				
Burn	40					
Blood( $\mu\text{g/L}$ )			$1.72 \pm 0.21^b$	$2.76 \pm 0.25^b$	$1.54 \pm 0.24^b$	$1.19 \pm 0.13^b$
Intestine( $\text{ng/g}$ )			$1.80 \pm 0.21^b$	$2.34 \pm 0.18^b$	$1.68 \pm 0.15^b$	$1.42 \pm 0.16^b$
Treatment	40					
Blood( $\mu\text{g/L}$ )			$0.84 \pm 0.16^{da}$	$1.46 \pm 0.27^{db}$	$0.93 \pm 0.18^{ca}$	$0.71 \pm 0.15^c$
Intestine( $\text{ng/g}$ )			$0.67 \pm 0.07^{da}$	$1.24 \pm 0.13^{dbaa}$	$0.83 \pm 0.12^{da}$	$0.64 \pm 0.08^{da}$

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs control; <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$  vs burn group.

**Table 2 Changes of intestinal mucosal permeability ( $\text{mL} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ,  $\bar{x} \pm s$ )**

Group	n		After burn (h)			
			6	12	24	48
Control	10	$0.07 \pm 0.02$				
Burn	40		$0.33 \pm 0.14^b$	$0.58 \pm 0.18^b$	$0.21 \pm 0.07^b$	$0.13 \pm 0.04^b$
Treatment	40		$0.19 \pm 0.05^{db}$	$0.27 \pm 0.06^{db}$	$0.10 \pm 0.04^{da}$	$0.08 \pm 0.03^c$

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs control; <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$  vs burn group.

**Table 3 Levels of plasma endotoxin ( $\text{ng/L}$ ,  $\bar{x} \pm s$ )**

Group	n		After burn (h)			
			6	12	24	48
Control	10	$34 \pm 8$				
Burn	40		$93 \pm 28^b$	$129 \pm 32^b$	$90 \pm 22^b$	$59 \pm 16^b$
Treatment	40		$57 \pm 15^{ca}$	$66 \pm 13^{db}$	$50 \pm 10^{da}$	$43 \pm 8^c$

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs control, <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$  vs burn group.

## DISCUSSION

The pathogenesis of enterogenic endotoxemia remains poorly understood. It is considered that the main cause is injury of intestinal mucosal barrier. Under physiological condition, the intestinal mucosa functions as a major local defense barrier preventing intestinal bacteria and endotoxin from invading distant organs and tissues. However, under the circumstances of trauma, shock and sepsis, the impairment of intestinal barrier may result from ischemic damage of the intestinal mucosa, the bacteria and endotoxin in gastrointestinal tract can pass through the intestinal barrier to mesenteric lymph nodes and systemic organs, resulting in enterogenic sepsis (endotoxemia)<sup>[6,7]</sup>. There is increasing evidence that enterogenic sepsis may play an important role in the development of systemic infection as well as multiple organ failure<sup>[8,9]</sup>. The present study results show that increased intestinal permeability after burn is an important cause of enterogenic endotoxemia.

PAF is a phospholipid mediator released from stimulated leukocytes, platelets, endothelial and mast cells<sup>[10,11]</sup>. It has been regarded as an important endogenous mediator of shock, sepsis and multiple organ failure. Our study demonstrated that PAF contents in blood and intestinal tissue after burn were all significantly increased and were positively correlated with the increase of intestinal permeability and plasma endotoxin. Treatment with PAF antagonist can significantly decrease intestinal permeability and plasma endotoxin. These suggest that PAF is involved in the process of increasing the intestinal permeability after burn and is also an important factor leading to enterogenic endotoxemia.

Previous studies showed that endotoxin can stimulate directly or indirectly macrophages and endothelial cells to release PAF, which also

mediates some endotoxin-induced pathologic processes of multiple organ injury<sup>[12,13]</sup>. It is suggested that a PAF-endotoxin positive feedback relationship existed in the body. Therefore, administration of PAF antagonists has an important effect on preventing and treating enterogenic endotoxemia.

## REFERENCES

- 1 Jones WG, Barber AE, Minei JP, Fahey III TJ, Shires III GT, Shires GT. Differential pathophysiology of bacterial translocation after thermal injury and sepsis. *Ann Surg*, 1991;214:24-30
- 2 LeVoyer T, Cioffi WG, Pratt L, Shippee R, McManus WF, Mason AD, Pruitt BA. Alterations in intestinal permeability after thermal injury. *Arch Surg*, 1992;127:26-30
- 3 Gianotti L, Braga M, Vaiani R, Almondo F, DiCarlo V. Experimental gut-derived endotoxaemia and bacteraemia are reduced by systemic administration of monoclonal anti LPS antibodies. *Burns*, 1996;22:120-124
- 4 Anderson BO, Bensard DD, Harken AH, Colorado D. The role of platelet activating factor and its antagonists in shock, sepsis and multiple organ failure. *Surg Gynecol Obstet*, 1991;172:415-424
- 5 Beyer AJ, Smalley DM, Shyr YM, Wood JG, Cheung LY. PAF and CD18 mediate neutrophil infiltration in upper gastrointestinal tract during intra-abdominal sepsis. *Am J Physiol*, 1998;275:G467-G472
- 6 Wilmore DW, Smith RJ, O'Dwyer ST, Jacobs DO, Ziegler TR, Wang XD. The gut: a central organ after surgical stress. *Surgery*, 1988;104:917-922
- 7 Fink MP. Gastrointestinal mucosal injury in experimental models of shock, trauma, and sepsis. *Crit Care Med*, 1991;19:627-640
- 8 Fukushima R, Gianotti L, Alexander JW, Pyles T. The degree of bacterial translocation is a determinant factor for mortality after burn injury and is improved by prostaglandin analogs. *Ann Surg*, 1992;216:438-445
- 9 Moore FA, Moore EE. Evolving concepts in the pathogenesis of postinjury multiple organ failure. *Surg Clin North Am*, 1995;75:257-277
- 10 Snyder F. Platelet activating factor and related acetylated lipids as potent biologically active cellular mediators. *Am J Physiol*, 1990;259:C697-C708
- 11 Kubes P, Arfors KE, Granger DN. Platelet-activating factor induced mucosal dysfunction: role of oxidants and granulocytes. *Am J Physiol*, 1991;260:G965-G971
- 12 Torley LW, Pickett WC, Carroll ML, Kohler CA, Schaub RE, Wissner A. Studies of the effect of platelet activating factor antagonist, CL184,005, in animal models of Gramnegative bacterial sepsis. *Antimicrob Agents Chemother*, 1992;36:1971-1977
- 13 Fletcher JR, Disimone AG, Earnest MA. Platelet activating factor receptor antagonist improves survival and attenuates eicosanoid release in severe endotoxemia. *Ann Surg*, 1990;211:312-316

Edited by You DY and Zhu LH  
proofread by Sun SM