The effects of PAF antagonist on intestinal mucosal microcirculation after burn in rats

Pei Wu Yu1, Guang Xia Xiao2, Xiao jian Qin2, Li Xin Zhou2 and Zi Qiang Wang1

Subject headings platelet activating factor; intestinal mucosa; intramucosal PH; burn

Yu PW, Xiao GX, Qin XJ, Zhou LX, Wang ZQ. The effects of PAF antagonist on intestinal mucosal microcirculation after burn in rats. World J Gastroenterol, 2000; 6(6):906-908

INTRODUCTION
Gut originated infection (GOI) has been recognized as a potential factor for postburn irreversible shock, early sepsis and multiple system organ failure[1-5]. The intestinal mucosal barrier injury has been implicated as the cause of postburn GOI[6-8]. However, pathogenesis of the lesion is not well known. Platelet activating factor (PAF), an endogenous phospholipid mediator, has recently been proposed as an important mediator of postburn intestinal mucosal barrier injury and gut originated infection[9-11]. But the mechanism of PAF is not well defined. In this study, we have evaluated sequential hemodynamic changes in the intestinal mucosa after burn injury and investigated the role of PAF by assessing whether pretreatment against intestinal mucosal hemodynamic disturbance and pathologic damage, so as to further explore the role and its mechanism of PAF in postburn intestinal mucosal barrier injury.

MATERIALS AND METHOD
Animals
Wistar rats, male or female, weighing 220 ± 30 g were used. They were provided by Animal Laboratory, Institute of Burn Research, Third Military Medical University.

Experimental design
Animals were randomly divided into three groups: group 1 (n=10) served as a control with sham, burn injury; group 2 (n=40), burned rats that had undergone 30% TBSA III° burn; group 3 (n=40), rats that received PAF antagonist WEB2170 (5mg/kg) by intraperitoneal injection immediately after burn and repeated every 8 hours[12]. WEB2170 was provided by Boeringer Ingelheim Pharmaceuticals Inc, Federal Republic of Germany. The index was observed on postburn 6, 12, 24, and 48 hours.

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

Measurement of intestinal mucosal blood flow
The intestinal mucosal blood flow was directly measured with a laser-Doppler flowmeter[13], made by Nakai university. The unit was expressed as mv.

Measurement of intestinal intramucosal PH (PHi)[14,15]
Rats were anesthetized after fasted overnight, a midline abdominal incision was made. Fifteen cm segments of ileum were isolated, cannulated proximally and distally, and 3mL saline was injected into the ileal segment. After 30min, 1mL intestinal perfusion and 1mL arterial blood were collected for measurement of Pco2 in intestinal perfusion and [HCO3-] in arterial blood. The intestinal mucosal PHi was calculated from the Henderson-Hasselhach equation:

PHi=6.1+log(Pco2×0.0307)

Measurement of intestinal water content[16] Five cm segments of ileum were excised and weighed for wet weight, and placed in to 140°C oven for 4 hours and weighed again for dry weight. The intestinal water content was calculated using the following formula:

Water content=(wet weight-dry weight/wet weight)×100%

Histologic examination of intestinal mucosa The ileum was fixed in 10% formalin and processed by the routine techniques. Specimens were stained with hematoxylin and eosin and examined histologically under light microscope.
Statistical analysis
All data were expressed as x±s, and statistical analyses were made using Student’s t test.

RESULTS
The changes of intestinal mucosal blood flow
The intestinal mucosal blood flow began to decrease significantly on postburn 6h and became lowest on postburn 12h as compared with control group. In PAF-antagonist treatment group, the intestinal mucosal blood flow was significantly increased compared with burn group (Table 1).

The changes of intestinal mucosal Phi
The intestinal mucosal Phi in burn group was significantly lower than in control group on postburn 6h, 12h, and 24h, but not on postburn 48h. In PAF antagonist treatment group, intestinal mucosal Phi was significantly increased compared with burn group (Table 2).

The changes of intestinal water content
The changes of intestinal water content on postburn 6h and 48h were not significantly different, but on postburn 12h and 24h, it was significantly increased as against burn group. The intestinal tissue water content in treatment group on 12h and 24h was significantly lower than in the burn group (Table 3).

The histologic changes of intestinal mucosa
In the burn group, extensive vascular congestion and edema were noted in ileal mucosa. Subepithelial space at the tip of the villi was developed, and lacteals were dilated. The degenerative fragmentation and atrophy of mucosal villi were apparent on postburn 12h and 24h. These changes were significantly reduced in PAF antagonist treatment group.

DISCUSSION
The intestinal mucosal blood flow is an important factor for maintaining the structure and function of intestinal epithelial cells[17-19]. Some studies have indicated that gastrointestinal mucosal blood flow significantly decreased following early burn injury[20-22]. Binnaka et al[23] reported that gastric mucosal blood flow fell rapidly to 40% of normal value on postburn 2h on a rat model with 30% TBSA III° burn. Tokay et al[24] reported that intestinal blood flow decreased significantly to 25% -30% of the baseline on 2h and 4h of the early postburn phase, and during the late phase at 48h to 30% of the baseline again on a pig model with 40% TBSA III° burn. The results showed that intestinal mucosal blood flow significantly decreased to 65% of normal value on postburn 6h to 46% on postburn 12h, to 68% and 82% on postburn 24h and 48h. The intestinal mucosal ischemia following burn was proved again. The decrease in the intestinal mucosal blood flow caused a decrease in oxygen delivery (DO₂) and a marked increase in oxygen consumption (VO₂) in intestinal epithelial cells[25,26]. When DO₂ fell below a critical level, further decrease in DO₂ can induce anaerobic metabolism and decrease in cells PH, causing the damage of intestinal epithelial cells[27,28]. The results also showed that the intestinal mucosal Phi was significantly decreased following burn injury compared with control group. It is suggested that hypoxia in intestinal epithelial cells occurs following burn.

Table 1 Changes of the intestinal mucosal blood flow (mv, x±s)

| Groups   | n  | Postburn          | 6h       | 12h       | 24h       | 48h       |
|----------|----|-------------------|----------|----------|----------|----------|
| Control  | 10 | 46.5±3.01         |          |          |          |          |
| Burn     | 40 | 30.60±3.08b       | 21.85±2.94b | 31.85±2.72b | 58.56±3.11b |          |
| Treatment| 40 | 40.22±2.86a       | 37.1±2.90b | 42.06±2.14a | 45.89±4.51c |          |

*P<0.05, aP<0.01 vs Control; bP<0.05, dP<0.01 vs Burn.

Table 2 Changes of the intestinal mucosal Phi (x±s)

| Groups   | n  | Postburn          | 6h       | 12h       | 24h       | 48h       |
|----------|----|-------------------|----------|----------|----------|----------|
| Control  | 10 | 7.419±0.058       |          |          |          |          |
| Burn     | 40 | 7.217±0.085b      | 7.316±0.067b | 7.347±0.016e | 7.432±0.046 |          |
| Treatment| 40 | 7.326±0.087a      | 7.406±0.113c | 7.374±0.148c | 7.435±0.035 |          |

*P<0.05, aP<0.01 vs Control; bP<0.05, dP<0.01 vs Burn.

Table 3 Changes of the intestinal tissue water content (% x±s)

| Groups   | n  | Postburn          | 6h       | 12h       | 24h       | 48h       |
|----------|----|-------------------|----------|----------|----------|----------|
| Control  | 10 | 76.02±2.97        |          |          |          |          |
| Burn     | 40 | 76.21±4.16        | 80.43±2.78c | 79.89±2.60c | 76.97±1.91 |          |
| Treatment| 40 | 75.83±2.67        | 76.65±2.14c | 76.47±1.43c | 76.14±1.56 |          |

*P<0.05, vs Control; dP<0.05, vs Burn.
PAF is a phospholipid mediator released from stimulated leukocyte, platelets, endothelial cells and mast cells, etc. PAF has a potent vasoactive effect, intravenous infusion of PAF can induce hypotension and extensive vasoconstriction in heart, lung, brain and gastrointestinal, and a marked increase of vascular permeability as well. PAF-induced responsiveness was significantly attenuated by PAF antagonist. It was reported that PAF antagonist can significantly reduce intestinal mucosal ischemia and pathological damage caused by endotoxin shock and hemorrhagic shock. In this study, treatment with antagonist WEB2170 for the scalded rats could significantly increase the intestinal mucosal blood flow and PHi, decrease intestinal tissue water content and alleviate the pathological damage of intestinal mucosa. Conclusion can be drawn that PAF is one of the important factors causing postburn disturbance of intestinal mucosal microcirculation.

REFERENCES

1. Wilmore DW, Smith RJ, O’Dwyer ST, Jacobs DO, Ziegler TR, Wang XD. The gut: A central organ after surgical stress. Surgery, 1988; 104:97-102
2. Moore FA, Moore EE. Evolving concepts in the pathogenesis of postinjury systemic inflammatory response syndrome. Surg Clin North Am, 1995;75:257-261
3. Gianotti L, Braga M, Vaiani R, Almondo F, Di Carlo V. Experimental gut-derived endotoxaemia and bacterial translocation in rats with intestinal ischemia. J Surg Res, 1993;57:197-204
4. Epstein MD, Banducci DR, Manders EK. The role of the gastrointestinal tract in the development of burn sepsis. Plast Reconstr Surg, 1992;90:524-531
5. Wu CT, Li ZL, Xiong DX. Relationship between enteric microecologic dysbiosis and bacterial translocation in acute necrotizing pancreatitis. World J Gastroenterol, 1998;4:242-245
6. Yi JH, Ni RY, Luo DD, Li SL. Intestinal flora translocation and overgrowth in upper gastrointestinal tract induced by hepatic failure. World J Gastroenterol, 1999;5:327-329
7. Baron P, Traber LD, Traber DL, Nguyen T, Hollyouk M, Heggars JP, Herndon DN. Gut failure and translocation following burn and sepsis. J Surg Res, 1994;57:197-204
8. Epstein MD, Banducci DR, Manders EK. The role of the gastrointestinal tract in the development of burn sepsis. Plast Reconstr Surg, 1992;90:524-531
9. Ruan CP, Wang YH, Wang LG, Wang YX. Changes of neutrophil and endotoxin in rats with intestinal ischemia. China Natl J New Gastroenterol, 1996;2:200-202
10. Yu PW, Xiao GX, Fu WL, Qiu XJ, Zhou LX. Role of platelet activating factor in intestinal mucosal barrier injury after burn. Zhonghua Waike Za Zhi, 1995;33:393-395
11. Yu PW, Xiao GX, Fu WL, Qiu XJ, Yuan JC. The roles of platelet activating factor in intestinal mucosal barrier injury after burn. Zhonghua Waike Za Zhi, 1996;33:65-69
12. Yu PW, Xiao GX, Fu WL, Yuan JC, Zhou LX, Qiu XJ. Pathogenetic effects of platelet activating factor on enteric endotoxemia after burn. World J Gastroenterol, 2000;6:451-453
13. Zhou W, Levine BA, Olson MS. Platelet-activating factor: a mediator of pancreatic inflammation during cerulein hyperstimulation. Am J Pathol, 1993;142:1504-1512
14. Diedel LN, Dulchavy SA, Brown WJ. Splanchnic ischemia and bacterial translocation in the abdominal compartment syndrome. J Trauma, 97:43:832-855
15. Wang XD, Wang Q, Andersson R, Bengmark S. Intramucosal pH and oxygen extraction in the gastrointestinal tract after major liver resection in rats. Eur J Surg, 1993;159:81-87
16. Schluchter E, Lyberg T. Monitoring of tissue oxygenation in shock: an experimental study in rats. Crit Care Med, 1995;23:1703-1710
17. Li CZ, Li A, Wang SL, You ZY, Tang CG. Peroxidation of the small intestine and its effect on absorption of amino acids in burned rats. Zhonghua Zhengxing Shaoshang Waike Za Zhi, 1990;6:53-56
18. Chen DC, Yang XY, Zhang XY, Chen XY. Protective effect of rhubarb on barrier of intestinal mucosa. China Natl J New Gastroenterol, 1997;1:81-85
19. Qin HL, Cui HG, Zhang CH, Wu DW, Chu XP. Effects of glutamine on structure and function of gut in endotoxemic rats. China Natl J New Gastroenterol, 1996;2:69-72
20. Tokay R, Zeigler ST, Traber DL, Stothert JC, Loick HM, Heggars JP, Herndon DN. Postburn gastrointestinal vasoconstriction increases bacterial and endotoxin translocation. J Appl Physiol, 1993;74:1521-1527
21. Arevalo JM, Lorente JA, Esteban A. The balance between oxygen supply and demand in the intestine can be assessed by measuring the difference between arterial and intramuscular PCO2, estimated by means of a gastric tonometer. J Trauma, 1998;44:569
22. Zhu L, Yang ZC, Li A, Cheng DC. Reduced gastric acid production in burn shock periods after resuscitation with hypertonic saline dextran. J Trauma, 1996;42:139-140
23. Binnaka T, Yamaguchi T, Kubota Y, Fujimura K, Tani K, Kitagawa S, Mizuno T, Inoue K. Burn-induced gastric mucosal hemodynamic disturbance in the rat. Role of platelet activating factor. Scand J Clin Lab Invest, 1992;27:89-92
24. Tokay R, Loick HM, Traber DL, Heggars JP, Herndon DN. Effects of thromboxane synthetase inhibition on postburn mesenteric vascular resistance and the rate of bacterial translocation in a chronic porcine model. SGO, 1992;174:125-132
25. Demling RH, Knox J, Youn YK, LaLonde C. Oxygen consumption early postburn becomes oxygen delivery dependent with the addition of smoke inhalation injury. J Trauma, 1992;32:593-598
26. Tokay R, Zeigler ST, Kramer GC, Rogers CS, Heggars JP, Traber DL, Herndon DN. Effects of hypertonic saline dextan resuscitation on oxygen delivery, oxygen consumption, and lipid peroxidation after burn injury. J Trauma, 1992;32:704-712
27. Haglund U, Rasmussen I. Oxygenation of the gut mucosa. Br J Surg, 1983:90-953-956
28. Li SZ, Tan XH. Effects of Astragalus Membranaceus on oxygen consumption of intestine. China Natl J New Gastroenterol, 1997;3:182-184
29. Huang Q, Wu M, Meininger C, Kelly K, Yuan Y. Neutrophil dependent augmentation of PAF induced vasoconstriction and albumin flux in corneal arteries. Am J Physiol, 1995;275:H141-H147
30. Cheng DY, Chen WB, Yang XD. The level of platelet activating factor in blood of rats with hypoxic pulmonary. Huaxi Yi Ke Daxue Xuebao, 1996;27:139-142
31. Sun ZW, Wang XD, Lasson A, B-riesson A, Leveque P, Haraldsen P, Andersson R. Roles of platelet-activating factor, interleukin-1 β and interleukin-6 in intestinal barrier dysfunction induced by mesenteric arterial ischemia and reperfusion. J Surg Res, 1999;82:90-100
32. He Y, Wang YM, He Y, Yuan FY, Ding J. Effect of PAF on the cultured hepatic cells in vitro. Shiji Huaren Xiohua Zazhi, 1992;7:894-895
33. Anderson BO, Bensard DD, Harken AH. The role of platelet activating factor and its antagonists in shock sepsis and multiple organ failure. SGO, 1991;172:415-424
34. Beyer AJ, Smalley DM, Shyr YM, Wood JG, Chueng LY. PAF and CD18 mediate neutrophil infiltration in upper gastrointestinal tract during intra-abdominal sepsis. Am J Physiol, 1998;275:G467-G472
35. Torley LW, Pickett WC, Carroll ML, Kohler CA, Schaub RE, Wissner A, Dejoy SQ, Oronsky AL, Kerwar SS. Studies of the effect of a platelet-activating factor antagonist, CL 184.005, in animal models of gram-negative bacterial sepsis. Antimicro Agents Chemother, 1992;36:1977-1977
36. Wallace JL, Hogaboam CM, Mcknight GW. Platelet activating factor mediates gastric damage induced by hemorrhagic shock. Gastroenterol, 1992;5:140-G141
37. Abu Zidan FM, Walther S, Lennquist S. Role of platelet-activating factor antagonism in posthemorrhage septic shock. J Trauma, 1996;41:634-640
38. Abu-Zidan FM, Walther S, Lennquist S. Role of platelet-activating factor antagonism in hemorrhagic shock in pigs. Eur Surg Res, 1995;27:379-388

Edited by Ma JY