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

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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 g±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 hydrocloride, 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℃ 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 Pco_2 in intestinal perfusion and [HCO_3^-] in arterial blood. The intestinal mucosal PHi was calculated from the Henderson-Hasselhach equation:

$$PHi=6.1+\log(Pco_2\times 0.0307)$$

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

$$\text{Water content}=(\text{wet weight}-\text{dry weight}/\text{wet weight})\times 100\%$$

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.

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Statistical analysis

All data were expressed as $x \pm s$, 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. Tokyay *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 (DO_2) and a marked increase in oxygen consumption (VO_2) in intestinal epithelial cells[25,26]. When DO_2 fell below a critical level, further decrease in DO_2 can induce anaerobic metablism 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, $\bar{x}\pm s$)

Groups	*n*		Postburn			
			6h	12h	24h	48h
Control	10	46.55±3.01				
Burn	40		30.60±3.08[b]	21.85±2.94[b]	31.85±2.72[b]	58.56±3.11[a]
Treatment	40		40.22±2.86[da]	37.1±2.90[db]	42.06±2.14[da]	45.89±4.51[c]

[a]P<0.05, [b]P<0.01 *vs* Control; [c]P<0.05, [d]P<0.01 *vs* Burn.

Table 2 Changes of the intestinal mucosal PHi ($\bar{x}\pm s$)

Groups	*n*		Postburn			
			6h	12h	24h	48h
Control	10	7.419±0.058				
Burn	40		7.217±0.085[b]	7.316±0.067[b]	7.347±0.016[a]	7.432±0.046
Treatment	40		7.326±0.087[da]	7.406±0.113[c]	7.374±0.148[c]	7.435±0.055

[a]P<0.05, [b]P<0.01 *vs* Control; [c]P<0.05, [d]P<0.01 *vs* Burn.

Table 3 Changes of the intestinal tissue water content (%, $\bar{x}\pm s$)

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

[a]P<0.05, *vs* Control; [c]P<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[29,30]. intravascular infusion of PAF can induce hypotension and extensive vasoconstriction in heart, lung, brain and gastrointestine, and a marked increase of vascular permeability as well[31,32]. PAF-induced responsiveness was significantly attenuated by PAF antagonist[33,34]. It was reported that PAF antagonist can significantly reduce intestinal mucosal ischemia and pathological damage caused by endotoxin shock and hemorrhagic shock[35-38]. 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 drown that PAF is one of the important factors causing postburn disturbance of intestinal mucosal microcirculation.

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