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Effects of platelet–activating factor on rat mesenteric microcirculation[1]

LI Shao–Hua, GONG Xiao–Qi, FEI Xia, WU Zhong–Li (*Department of Pathophysiology, Second Military Medical University, Shanghai 200433, China*)

ABSTRACT The actions of platelet–activating factor (PAF) on rat mesenteric microcirculation were studied by laser Doppler microscopy *in vivo*. PAF 0.2 – 0.6 μg / kg iv produced a dose–related decrease in the blood flow velocity and an increase in the diameters of the mesenteric arterioles and venules. These responses were completely reversed by pretreatment with PAF receptor antagonist SRI 63441. The results suggest that PAF may be a mediator of microcirculatory disturbances in the disease conditions associated with excessive PAF release.

KEY WORDS platelet activating factor; microcirculation; mesenteric arteries; mesenteric veins; flowmeters; rat

Platelet activating factor (PAF, 1–*O*–alkyl–2–acetyl–*L*–3–phosphatidylcholine) is found to be increased in the blood and tissues of animals with endotoxic shock, anaphylaxis, serum sickness, and local inflammatory reactions[1]. When iv into healthy animals, PAF produces a vascular permeability increment, systemic hypotension, bronchoconstriction, activation of intra– vascular leukocytes and platelets, and cardiac abnormalities[2]. However, few studies have been done on the effects of PAF on microvascular beds examined *in vivo*. The purpose of this study is to investigate the effects of PAF on rat mesenteric microcircula– tion and whether these effects are mediated by PAF receptor.

MATERIALS AND METHODS

Synthetic PAF was purchased from Sigma Chemical Company. Compound SRI 63441, *DL*–erythro–hexitol, 2,5–anhydro–3,4–di–deoxyoctadecylcarbamate–2–quinoli–nioethyl hydrogen phosphate, hydroxide, inner salt, an antagonist of PAF, was kindly supplied by Dr DA Handley at Sandoz Research Institute. PAF and SRI 63441 were dissolved for injection in 66.7 mmol/L phosphate buffer (PBS, pH 7.4) containing 0.25% bovine serum albumin.

Mesenteric microcirculation *in vivo* Sprague–Dawley rats ♀, ♂, weighing 196 ± SD 13 g were anesthetized with urethane (1.2 g / kg, im). The left common carotid artery was cannulated and connected with a CYS pressure transducer and an FY–2 blood pressure amplifier (Chengdu Instrument Factory). The left cervical vein was cannulated for injection. The small intestine was pulled out through a midline incision on the abdominal wall into a chamber and bathed in normal saline solution kept at 38℃ with a thermostatic apparatus. The microvasculature in the mesentery was observed under the intravital microscope with a large stage. The microcirculation was monitored through a TV camera (Shanghai № 822 Factory). The blood flow velocities in the arterioles and venules were measured with a laser Doppler microscope (Ningbo Optical Instrument Factory) and recorded simultaneously with arterial blood pressure with a LM 14164 self–balanced recorder (Dahua Instrument Factory, Shanghai) . The changes in the diameter of the microvessels were analyzed on a TV screen with a light bar calibration. SRI 63441 or PBS was given 5 min before PAF.

Statistical analysis Analysis of variance with two–way layout was used for comparisons between different groups before and after PAF administration.

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Tab 1. Dose-related changes of rat mean arterial pressure (MAP) and mesenteric microcirculation 30 s after platelet-activating factor (PAF) challenge. $n = 8$. $\bar{x} \pm$ SD. $^{***}P < 0.01$ *vs* control.

Parameter	Control	Platelet activating factor (μg / kg)		
		0.2	0.4	0.6
MAP (kPa)	16.47 ± 1.28	13.93 ± 1.28***	12.85 ± 2.05***	12.07 ± 1.48***
MABFV (mm / s)	0.99 ± 0.37	0.66 ± 0.13***	0.57 ± 0.14***	0.48 ± 0.18***
MVBFV (mm / s)	0.54 ± 0.10	0.46 ± 0.06***	0.36 ± 0.14***	0.33 ± 0.15***
MAD (μm)	50.5 ± 4.5	54.3 ± 5.1***	56.5 ± 6.2***	57.8 ± 6.4***
MVD (μm)	68.0 ± 6.5	73.3 ± 6.7***	74.5 ± 10.2***	79.0 ± 9.2***

MABFV: mesenteric arteriolar blood flow velocity; MVBFV: mesenteric venular blood flow velocity; MAD: mesenteric arteriolar diameter; MVD: mesenteric venular diameter.

RESULTS

Effect of PAF on mean arterial blood pressure PAF 0.2 ~ 0.6 μg / kg iv caused a dose-related decrease in rat mean arterial blood pressure (MAP) as shown in Tab 1. The MAP reduction peaked at 0.5 min and was followed by a 3 min recovery period to preinjection values after PAF administration. Before a challenge with PAF 0.4 μg / kg , SRI 63441 1 mg / kg iv could completely abolish PAF-induced hypotension (Tab 2).

Tab 2. Antagonistic effects of SRI 63441 on hypotension and the changes of mesenteric microcirculation induced by PAF injection in rats. $n = 8$. $\bar{x} \pm$ SD. $^{***}P < 0.01$ *vs* PAF 0.4 μg / kg group, $^{+}P > 0.05$ *vs* control.

PAF (μg / kg)	0	0.4	0.4
SRI (mg / kg)	0	0	1.0
MAP (kPa)	16.47 ± 1.28	12.85 ± 2.05	16.38 ± 1.07***+
MABFV (mm / s)	0.99 ± 0.37	0.57 ± 0.14	0.89 ± 0.42***+
MVBFV (mm / s)	0.54 ± 0.10	0.36 ± 0.14	0.56 ± 0.13***+
MAD (μm)	50.5 ± 4.5	56.5 ± 6.2	52.5 ± 6.7***+
MVD (μm)	68.0 ± 6.5	74.5 ± 10.2	70.0 ± 7.1***+

Effect of PAF on mesenteric microcirculation Immediately after iv PAF, there was an increase in leukocytes sticking to and rolling along the mesenteric venules. The phenomenon lasted more than 5 min. No "white thrombli" in the microvessels and focal hemorrhage were observed. The erythrocyte flow velocity as measured by laser Doppler technique in the arterioles and venules was decreased in a dose-related manner after PAF administration compared with the baseline and control values. The arterioles and venules were simultaneously dilated in a dose-related manner (Tab 1). The maximal reduction of blood flow velocity and dilation of the microvessels was at 0.5 min and returned to baselines 2 min after PAF injection. The axial flow of the erythrocyte disappeared before blood flow recovery. At a challenge with PAF 0.4 μg / kg , the blood flow velocity in the arterioles and venules decreased by 40.0% and 32.1%, respectively. And the internal diameters of the arterioles and venules increased by 10.8% and 10.0%, respectively. The decrease in blood flow velocity and the dilation of the microvessels were reversed by pretreatment with SRI 63441 (Tab 2). SRI 63441 itself had no evident effect on MAP and the mesenteric microcirculation.

DISCUSSION

PAF iv produced systemic hypotension and increased adherance of leukocytes to the endothelium in the mesenteric venules, which may contribute to the decrease of the blood flow velocity in mesenteric microvessels. The reduction of blood flow velocity led to the disappearance of erythrocyte axial flow which might further increase the blood flow resistance. These results suggested that under the disease conditions such as endotoxemia, exces-

sive PAF release from activated leukocytes and platelets might initiate microcirculatory disturbances and induce anaphylactic shock.

The mesenteric microvascular dilation may be a direct action of PAF on vasculature mediated by PAF receptor as the reflex response to systemic hypotension is vasoconstriction. If the most of peripheral microvasculature response to PAF challenge in the way of mesenteric microcirculation, the dilation of microvessels will play a part in PAF-induced systemic hypotension besides the decreased contractability of the heart[3] and the reduced blood volume after iv PAF[1].

Systemic inflammation induces the formation of "white thromboli" in microcirculation because of platelet aggregation. In the present study, "white thromboli" was not observed in the mesenteric microvasculature after PAF administration in rats, indicating that PAF would not activate rat platelets *in vivo*. These results are comparable with those of the previous study *in vitro*[4].

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血小板激活因子对活体大鼠微循环的影响

李少华、龚肖崎、黄 侠、吴中立 (第二军医大学病理生理学教研室, 上海 200433, 中国)

提要 应用激光多普勒微循环显微镜定量研究了血小板激活因子(PAF)对大鼠肠系膜微循环的影响. 发现 iv PAF 0.2, 0.4, 0.6 μg / kg 可引起剂量依赖性的动脉血压降低, 肠系膜微动脉和微静脉扩张及血流速度减慢, 白细胞对微静脉壁的粘附性增强. 上述效应可被 PAF 特异性受体拮抗剂 SRI 63-441 阻断.

关键词 血小板激活因子; 微循环; 肠系膜动脉; 肠系膜静脉; 流量计; 大鼠

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蝙蝠葛碱抑制卡西霉素刺激小鼠腹腔巨噬细胞释放血小板活化因子[1]

曾国钱、芮耀诚 (第二军医大学药学院药理教研室, 上海 200433, 中国)

Inhibitory effect of dauricine on platelet activating factor released from calcimycin-induced mouse peritoneal macrophages

ZENG Guo-Qian, RUI Yao-Cheng
(*Department of Pharmacology, College of Pharmacy, Second Military Medical University, Shanghai 200433, China*)

ABSTRACT The effects of dauricine (Dau) on the release of platelet activating factor (PAF) from mouse peritoneal macrophages stimulated by calcimycin (A-23187) was studied. The method of sodium [^{3}H] acetate incorporating into macrophages to synthesize PAF was set up for the first time. Calcimycin (0.2 μmol / L) significantly induced mouse peritoneal macrophages to utilize sodium [^{3}H] acetate to synthesize PAF. PAF released from macrophages medium fluid increased as the concentration of sodium [^{3}H] acetate increased. The maximal amount of PAF released from macrophages was attained by incubating macrophages with sodium [^{3}H] acetate (250 μmol / L) and calcimycin (2 μmol / L) over 30 min. Extracted by $CHCl_3$: CH_3OH : H_2O (2 : 2 : 1.8), seperated by thin

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