Original Article

Elevated Platelet Activating Factor Level in Ischemia-Related Arrhythmia and Its Electrophysiological Effect on Myocardium

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Abstract

Objective The mechanism through which platelet activating factor (PAF) induces cardiac electrical activity and arrhythmia is not well understood and previous studies have suggested a potential involvement of ion channels in its action. The present study was aimed to clarify the role of PAF in fatal arrhythmias following acute myocardia infarction (AMI) and the underlying mechanism.

Methods (1) Blood PAF levels were measured among 72 AMI patients at the time of diagnosis with AMI and 48 h later, and their electrocardiogram (ECG) was recorded continuously. (2) Ischemia simulation and surface electrocardiogram were conducted in 20 pigs and their PAF levels were measured. (3) PAF perfusion and standard microelectrode recording were performed on guinea pig papillary muscles.

Results In both humans and pigs, elevated PAF levels were detected in AMI and simulated ischemia, respectively, and even higher PAF levels were found when fatal arrhythmias occurred. In guinea pig myocardium, PAF induced a shortening of action potential duration at 90% level of repolarization (APD_{90})under non-ischemic conditions and a more pronounced shortening under early simulated ischemic conditions.

Conclusion AMI and ischemia are associated with increased PAF levels in humans and pigs, which are further raised when fatal arrhythmia follows. The effects of PAF on the myocardium may be mediated by multiple ion channels.

Key words: Platelet activating factor (PAF); Ischemia; Acute myocardial infarction; Fatal arrhythmia

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INTRODUCTION

Platelet activating factor (PAF) is a potent pro-inflammatory mediator with a broad range of biological activities. Quite a few types of cells, including cardiac myocytes and leukocytes, are capable of synthesizing and releasing PAF, which normally exists only in trace amounts[1]. However, its levels are significantly raised under certain pathological conditions. The action of PAF is mediated by specific receptors, which mobilize intracellular signal transduction systems to achieve individual effects[2]. In the cardiovascular system, PAF targets cardiac myocytes, smooth muscle cells

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and endothelial cells, and has been shown to be related to the pathogenesis of a number of morbid states, such as myocardial infarction, arrhythmia, stroke and sepsis[3].

Conflicting results concerning PAF levels in patients with myocardial infarction have been reported. Some noticed an increase while others saw no change in such cases[4-5]. In contrast, elevated PAF levels have been generally observed in several animal models of myocardial infarction[6-7]. It has been demonstrated that the cardiac effects of PAF include reduced coronary blood flow, reduced action potential duration and altered electrophysiological properties of the non-ischemic heart[8]. PAF can also induce arrhythmia under ischemic conditions[9]. The exact mechanism through which PAF generates cardiac electrical activity and arrhythmia remains poorly understood. Although it has been demonstrated that PAF at subnanomolar concentrations blocks the inwardly rectifying K channel in guinea pig ventricular myocytes[10], there is also evidence showing that depolarizing Na current may play a role in PAF-induced arrhythmia[9].

The present study was aimed at clarifying the role of PAF in fatal arrhythmias following acute myocardia infarction (AMI) and the underlying mechanism. Specifically, three sets of experiments were performed to investigate: (1) the relationship between PAF and fatal arrhythmias in AMI patients; (2) the relationship between PAF and fatal arrhythmias in a swine AMI model; (3) the electrophysiological effects of PAF on guinea pig papillary muscles.

METHODS AND PROCEDURES

This study was conducted at the China-Japan Friendship Hospital. The protocols of the study were approved by the ethics committee of the hospital.

PAF Measurement in AMI Patients with Fatal Arrhythmia

Seventy-two AMI patients (55 men and 17 women aged 45 to 68 with mean age of 48.5±3.5 years) were recruited in the study during the period from January 2009 to December 2009. All patients were admitted to the emergency department of the China-Japan Friendship Hospital within the first 12 h as of initial chest pain. Diagnosis of AMI was based on typical clinical symptoms, electrocardiography (ECG), troponin I, and coronary angiography. Informed consent was obtained before enrollment.

Blood samples for PAF measurement were collected when the diagnosis of AMI was made and 48 h later. ECG was monitored continuously. Fatal arrhythmia was defined as either ventricular fibrillation or ventricular tachycardia. The control group consisted of 35 healthy volunteers (28 men and 7 women aged 40 to 65 years with mean age of 45.3±2.8 years), whose blood samples were collected in the morning (after fasting for at least 8 h). Serum was prepared by centrifugation (3000 rpm, 5 min) without anticoagulants, and PAF measurement was made with an enzyme-linked immunosorbent assay kit for human PAF (RipadBio, USA).

PAF Measurement in a Swine AMI Model

Percutaneous transluminal coronary angioplasty was performed on pigs (weighing from 20.1 kg to 26.2 kg, n=20) of both sexes via percutaneous femoral puncture by using real time x-ray visualization. A balloon-tipped catheter was aligned to the opening of the left anterior descending coronary artery and inflated to occlude the artery for 1 h. The balloon-tipped catheter was then extracted and surface electrocardiogram was recorded for at least 1 hour. Blood samples were collected at both baseline and the time when the catheter was extracted. Serum was prepared by centrifugation (3000 rpm, 5 min) without anticoagulants and PAF was detected by an enzyme-linked immunosorbent assay kit for pig PAF (RipadBio, USA).

Measurement of Electrophysiological Effects of PAF on Guinea Pig Myocardia

Guinea pigs weighing 350 to 500 g were anesthetized and their hearts were removed immediately. Small papillary muscles of the left ventricles were isolated, placed in a 1 mL chamber and perfused with Tyrode's solution (in mmol/L NaCl 129, KCl 4, $NaHCO_3$ 20, NaH_2PO_3, 0.9, $CaCl_2$ 1.8, $MgCl_2$ 0.5 and D-glucose 10) at 5 mL/min. The temperature was maintained at 35±0.5 °C and the solution was gassed with 95% O_2 and 5% CO_2, at pH 7.35. The preparations were allowed to equilibrate for 2 h before testing. Driving stimuli were delivered to the preparations at 1 Hz through bipolar silver electrodes with rectangular pulses (1 ms duration and twice threshold strength). Transmembrane action potentials were recorded with glass microelectrodes with a resistance of 10-20

megaohms and filled with 3 mmol/L KCl. The microelectrodes were coupled to a high-impedance amplification system (MEZ-8300). Amplified signals were displayed on a storage oscilloscope (VC-11) and stored in a computer. To study the electrophysiological effects of PAF on guinea pig myocardia under non-ischemic conditions, preparations (n=8) were perfused with PAF (1 μmol/L) for 15 min and then washed with Tyrode's solution. Parameters that were recorded for PAF-induced electrophysiological effects included resting membrane potential (RMP), action potential amplitude (APA), and action potential duration at 90% level of repolarization (APD_{90}). To study the electrophysiological effects of PAF on guinea pig myocardia during ischemia and reperfusion, preparations (n=4) were perfused with PAF (1 μmol/L) constantly, while simulated ischemia was induced by perfusing the preparations with a hypoxic solution for 60 min. Then regular Tyrode's solution gassed with 95% O_2 and 5% CO_2 was infused to the chamber to simulate a reperfusion condition. The same electrophysiological parameters were recorded. Preparations (n=4) for the control group were subjected to the same simulated ischemia/reperfusion procedures without PAF perfusion. The hypoxic solution had nearly the same composition as Tyrode's solution, except that it was glucose-free and gassed with 95% N_2 and 5% CO_2. It had a pH of 6.8-7.0 and PO_2 of 6-9 mmHg. PAF (Sigma, USA) was dissolved in 50/50 water and dimethysulforid (DMSO) to make a 1 mmol/L stock solution.

Statistical Analysis

Values were expressed as means±SE. The statistical analysis was performed by using Student's t-test based on paired or unpaired observations. Statistical significance was defined as P<0.05. The Statistical Package for the Social Sciences (SPSS 13.0 for Windows, 2005, Chicago, IL) was used for all the analyses.

RESULTS

PAF Levels in AMI Patients

All patients received percutaneous coronary intervention after admission to the hospital and survived in the first 48 h. PAF levels (0.47±0.05 ng/mL) in AMI patients at baseline were higher than those (0.07±0.02 ng/mL) in the control group and became still higher 48 h later (3.65±0.15 ng/mL) (Table 1). Of the 72 patients, 40 developed fatal arrhythmias (mostly paroxysmal ventricular tachycardia) at varying time points within 48 h. PAF levels (4.72±0.16) in the AMI patients with fatal arrhythmias were higher than those (1.31±0.03 ng/mL) in the 32 patients who did not develop fatal arrhythmias 48 h after diagnosis of AMI (Table 2).

Table 1. PAF Levels in Patients with AMI

Group	PAF (ng/mL)	*P* Value
AMI at baseline (n=72)	0.47±0.05	<0.05[*]
AMI at 48 h (n=72)	3.65±0.15[#]	<0.05[#]
Control (n=35)	0.07±0.02	

Note. Student t-test was used for comparison between groups. [*]compared with control. [#]compared with control.

Table 2. PAF Levels in AMI Patients with or without Arrhythmia

Group	PAF (ng/mL)	*P* Value
AMI Patient with Arrhythmia	4.72±0.16	<0.05
AMI Patient without Arrhythmia	1.31±0.03	

Note. Student t-test was used for comparison between groups.

PAF Levels of Pigs in AMI Simulation

Compared with the baseline, PAF levels in pigs were higher in the ischemic phase (5.77±2.87 ng/mL *vs* 4.66±2.89 ng/mL, P=0.0005). During the ischemia/reperfusion simulation, fatal arrhythmias were observed in 13 pigs exhibiting elevated PAF levels, compared with those with no signs of fatal arrhythmia (1.92±1.34 ng/mL *vs* 0.28±0.74 ng/mL, P=0.003), (Table 3).

Table 3. PAF Levels in Pigs with Ischemia/ Reperfusion

Group	PAF (ng/mL)	*P* Value
Baseline	4.66±2.89	0.0005[*]
Ischemia	5.77±2.87	
Ischemia/Reperfusion with Arrhythmia (elevated PAF level)	1.92±1.34[§]	0.0003[#]
Ischemia/Reperfusion without Arrhythmia (elevated PAF level)	0.28±0.74[§]	

Note. [§]Values derived from subtraction of baseline values from measurements. [*]Paired t-test was used for comparison between groups. [#]Student t-test was used for comparison between groups.

Electrophysiological Effects of PAF on Guinea Pig Myocardia

When perfused with PAF, APD_{90} was markedly shortened (120.3±5.6 ms *vs* 144.0±6.3 ms, $P<0.001$) as compared with the baseline. The effects of PAF appeared about 5 min after the beginning of perfusion and reached the steady state within 10-15 min. After the preparations were perfused again with Tyrode's solution for 15-20 min, APD_{90} returned to baseline values. In contrast, APA and RMP remained largely unchanged during the entire course of perfusion with PAF and Tyrode's solution (Figure 1).

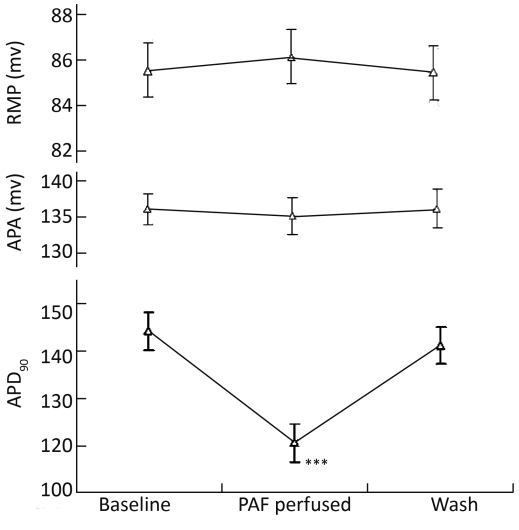

Figure 1. Effects of PAF on action potential characteristics. PAF: platelet activating factor (1 μmol/L). RMP: resting membrane potential; APA: action potential amplitude; APD_{90}: action potential duration at 90% level of repolarization, n=8, $^{***}P< 0.001$ *vs* control.

Simulated ischemia alone induced the shortening of action potential duration. With the addition of PAF, a further shortening of action potential duration was observed after 5 min under the simulated ischemic state (APD_{90}: 64.7%±2.7% *vs* APD_{90} at baseline: 93.3%±1.7%, $P<0.001$). However, PAF had no effect on action potential duration during the later ischemic phase. In contrast, PAF induced the lengthening of action potential duration during early reperfusion. With PAF, APD_{90} was longer at 10 and 15 min, respectively, after the reperfusion phase began (113.0%±3.8% *vs* 97.7%±3.0%, P=0.024; 118.5%±3.5% *vs* 99.4%±4.1%, P=0.020). Furthermore, APD_{90} was longer during early reperfusion than at baseline ($P<0.05$) (Figure 2).

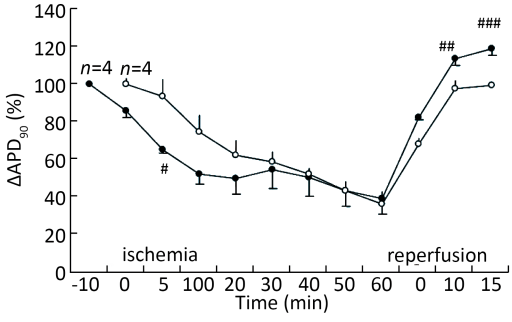

Figure 2. Effect of PAF on the course of APD during ischemia-reperfusion. (●):ischemia-reperfusion with PAF; (○): ischemia-reperfusion without PAF. $^{\#}P<0.001$, $^{\#\#}P=0.024$, $^{\#\#\#}P=0.020$ *vs* ischemia-reperfusion without PAF.

DISCUSSION

In the current study, we have demonstrated that PAF levels are elevated in AMI patients and higher levels of PAF are closely associated with fatal arrhythmias. In parallel with these findings, we have shown increased levels of PAF in pigs under ischemic conditions and even higher levels of PAF in pigs with fatal arrhythmias. Similar observations have been made by other investigators in human subjects and a number of animal species[4,6]. Although some studies have failed to establish a relationship between myocardial infarction and elevated PAF levels[5], there are other lines of evidence suggesting an important role of PAF in AMI and arrhythmia. For example, PAF receptor antagonists are capable of limiting PAF-induced hemodynamic effects and the size of infarct[11]. In addition, administration of PAF antagonists reduces the incidence of ventricular fibrillation and subsequent arrest during reperfusion caused by regional myocardial ischemia[12]. In murine ventricular myocytes, PAF (C-PAF), a stable analog of PAF, has been shown to generate abnormal automaticity, plateau phase arrest of the action potential and early after-depolarizations[13].

The types of cells that serve as sources of PAF include monocytes/macrophages, polymorphonuclear neutrophils (PMN), eosinophils, basophils, platelets and cardiac myocytes, most of which are prime participants in the inflammatory process[14]. In myocardial infarction, increased production of PAF by PMN is thought to be at least partially responsible for elevated PAF levels[15]. The coronary effects of

PAF have been found to be dose-dependent. In the low dose range (0.03-0.3 nmol/L), the effect of PAF on coronary blood flow is variable. In a higher range (1-10 nmol/L), PAF becomes a negative isotropic agent and induces increased coronary vascular resistance[3]. Moreover, PAF is also known to cause an increase in myocardial vascular permeability[16]. The mechanism through which PAF achieves its cardiovascular effects is not yet fully understood. Although it is known that PAF increases cytosolic Ca^{2+} and diacylglycerol levels, ultimately activating protein kinase C via a cell surface, G protein-linked receptor that initiates a signaling cascade involving activation of phospholipase C[17], the electrophysiology of PAF on membrane channels is poorly understood. Both K and Na channels have been shown to be involved in PAF-induced arrhythmogenic action[9-10]. For example, repolarization abnormalities and conduction arrhythmias induced by PAF in cardiac cells may be explained by alterations of inwardly rectifying background potassium channels[18] and inhibition of TWIK-related acid-sensitive K channels (TASK-1)[19]. In addition, PAF stimulates cardiac muscarinic potassium channels, via a PLA2-lipoxygenase dependent mechanism[20].

In order to better understand the action of PAF on cardiac electrophysiology, we attempted to characterize the electrophysiological effects of PAF on guinea pig myocardium. Under non-ischemic conditions, PAF induced a shortening of APD_{90}, whereas with simulated ischemia, PAF shortened APD_{90} even more. However, the effect wore out later. During the ischemic phase, PAF induced lengthened APD_{90}. The shortening of APD by PAF has been reported in other studies and may be related to reduction of both L-type calcium current and intracellular systolic calcium concentration[21]. The increase in intracellular Ca^{2+} may contribute to the negative effect of PAF on APD, since it reduces the driving force for Ca^{2+} ions and induces inactivation of slow inward current. Another mechanism may lie in increasing the time-dependent outward current[22-25]. However, the above explanations still fail to account for other experimental findings. For example, tetrodotoxin and lidocaine, blockers of other ionic channels, are capable of antagonizing the effect of PAF[22-25]. Therefore, aside from Ca^{2+} channels, other ionic channels could also be the targets for PAF.

As for the lengthening of action potential duration induced by PAF during reperfusion, it might possibly be associated with changing ATP levels in the tissue preparations. In our study, ATP levels presumably would decrease at the ischemic phase because of perfusion with the hypoxic and glucose-free solution; when Tyrode's solution gassed with 95% O_2 and 5% CO_2 was infused during the reperfusion phase, ATP levels would recover and consequently, the lengthening of APD was observed.

In conclusion, PAF levels are raised in patients with AMI and become even higher following the development of fatal arrhythmias. Similarly, increased levels of PAF were observed in pigs under ischemic conditions, which become higher with fatal arrhythmias. Different types of ion channels may be linked to the action of PAF on the myocardium.

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