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Effects of quercetin on platelet and reperfusion-induced arrhythmias in rats

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ABSTRACT The role of platelets in reperfusion arrhythmias (RA) and the efficacy of quercetin (Que) in preventing the arrhythmias were investigated in anesthetized rats. Platelet count (PC) was performed, the ultrastructure of platelets was scrutinized, and the levels of thromboxane B_2 (TXB_2) and 6-keto-prostaglandin F_1 alpha (Keto-$PGF_{1\alpha}$) in plasma were determined by radioimmunoassay (RIA). The pretreatment with Que (5 mg·kg^{-1}) 2 min prior to reperfusion inhibited RA. Que remarkably improved the ultrastructural deviation of platelet, inhibited the platelet aggregation and thromboxane A_2 (TXA_2) formation, and increased the prostacyclin (PGI_2) generation and the ratio of PGI_2/TXA_2. The decrease in PC and increase in TXB_2 level in plasma indicated the participation of platelets in the arrhythmogenic activity of ischemia and reperfusion. The results showed that Que produced a protective effect against RA probably through inhibiting the platelet aggregation, TXA_2 formation and increasing the PGI_2 generation.

KEY WORDS quercetin; arrhythmia; platelet aggregation; thromboxane A_2; thromboxane B_2; epoprostenol; 6-ketoprostaglandin F_1 alpha

Quercetin (Que) inhibited the platelet aggregation[1], the generation of oxygen free radicals[2,3], and the reperfusion arrhythmias[3,4]. Platelets were supposed to be a contributory factor in the pathogenesis of arrhythmias of ischemia and reperfusion[5]. The present study was to investigate the effects of Que on the platelet count, the concentration of TXB_2 and Keto-$PGF_{1\alpha}$, and the ultrastructural changes of platelets in the blood after reperfusion, so as to provide a further understanding of its anti-arrhythmic mechanism.

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MATERIALS AND METHODS

Rats Wistar ♂ rats were provided by the Animal Breeding Center, Suzhou Medical College.

Operative procedure Experiments were performed[1] on ♂ Wistar rats (210±*s* 18 g) anesthetized with ip sodium pentobital 45 mg·kg^{-1}. The trachea, and the right jugular vein were exposed. The rats were artificially ventilated with air at 60 strokes ·min^{-1} ventilated at 70 ml·kg^{-1} body weight, and the chest was opened by a parasternal incision. A silk ligature (1/0 Ethicon) was placed around the left anterier decending coronary (LAD). During a 15-min stabilization, any rat exhibiting ventricular arrhythmias was excluded from the study. A piece of plastic tube (5 mm long, 1 mm OD) was placed side by side to the coronary artery which was then occluded by applying tension upon the silk ligature. After 10 min the occlusion was released by removing the tube and the silk ligature. The rats were injected iv with either saline (control group) or test drugs 2 min before reperfusion. The ECG lead II was recorded before surgery, 30 s before occlusion, during occlusion, and continued for 10 min after the release of occlusion. The ventricular extrasystoles (VE), ventricular tachycardia (VT), and ventricular fibrillation (VF) were recorded.

Platelet count Blood was sampled with a plastic syringe and a 20 μl portion was immediately transferred into a plastic tube prefilled with 380 μl 1% ammonium oxalate. Platelets were counted in a Thoma Chamber under high power microscope.

Radioimmunoassay Blood 0.9 ml taken with a plastic syringe was transferred into a plastic tube prefilled with 0.1 ml 2% EDTA-Na_2. The tube was centrifuged for 10 min at 1200×*g*. The supernatant was frozen at −20℃. TXB_2 and Keto-$PGF_{1\alpha}$ were determined in paired assays with ^{125}I-TXB_2 and ^{125}I-Keto-$PGF_{1\alpha}$ RIA kits.

Ultrastructure of platelet The blood was collect-

ed in a bottle with 3.8% trisodium citrate. Platelet-rich plasma (PRP) was fixed in 4% glutaraldehyde and then in chilled osmic acid[6]. Observations were carried out using Hitachi H-600 electron microscope.

Chemicals Que was bought from Beijing Chemical Works (891005). The RIA kits for Keto-$PGF_{1\alpha}$ and TXB_2 were supplied by the Thrombosis and Hemostasis Research Unit, Suzhou Medical College. Other reagents were all of AR grade.

Statistical analysis Data are given in $\bar{x} \pm s$. Mortalities and incidence of arrhythmias were compared by chi-square tests. For changes in PC, TXB_2, and Keto-$PGF_{1\alpha}$, t test was used.

RESULTS

Mortality after reperfusion In the control group, 3/7 rats died 7—10 min after the release of occlusion and in Me_2SO group 3/7 rats died. In Que 0.2 mg·kg^{-1}, 2/7 rats died, at 1 mg·kg^{-1}, 5 mg·kg^{-1} and verapamil (Ver) 5 mg·kg^{-1}, all rats survived during the 10 min reperfusion period (Tab 1).

Tab 1. Effects of Que on reperfusion arrhythmias in anesthetized rats. $n=7$, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* saline.

	Dose/ mg·kg^{-1}	VT rats	VF rats	Duration of arrhythmias/s	Onset of VF/s	Rats died
Saline	—	7	7	193±67	12.6±4.7	3
1% Me_2SO	—	7[a]	7[a]	199±51[a]	12.4±5.1[a]	3[a]
Que	0.2	7[a]	7[a]	196±54[a]	11.6±6.0[a]	2[a]
	1.0	7[a]	2[b]	190±43[a]	23.5±0.7[b]	0[a]
	5.0	7[a]	0[c]	43±9[c]		0[a]
Ver	5.0	6[a]	0[a]	49±9[c]		0[a]

Reperfusion-induced arrhythmias The reperfusion-induced arrhythmias developed 10—20 s after the release of LAD occlusion and started usually as VE or VT followed by VF, which reversed automatically into sinus rhythm or into PVCs again. In the groups with Que 0.2 mg·kg^{-1}, Me_2SO, and the control group, all rats manifested VF. Que 1 mg·kg^{-1}, reduced VF to 29% (2/7); Que and Ver 5 mg·kg^{-1} prevented completely reperfusion-induced VF, and the duration of the arrhythmias shortened significantly ($P<0.01$). Que 1 mg·kg^{-1} obviated the onset of VF ($P<0.05$).

PC after reperfusion The control level of PC was $386 \pm 17 \times 10^9 \cdot L^{-1}$. PC lessened during occlusion and reperfusion in saline-treated rats ($P<0.05$, Fig 1). Que 5 mg·kg^{-1} increased the PC by 1.5% above the control value. PC in rats treated with 5 mg·kg^{-1} Que and Ver was higher *vs* that in the control group ($P<0.05$). Que 1 mg·kg^{-1} tended to increase the PC ($P>0.05$). In other groups no difference was seen *vs* the control group ($P>0.05$, Tab 2).

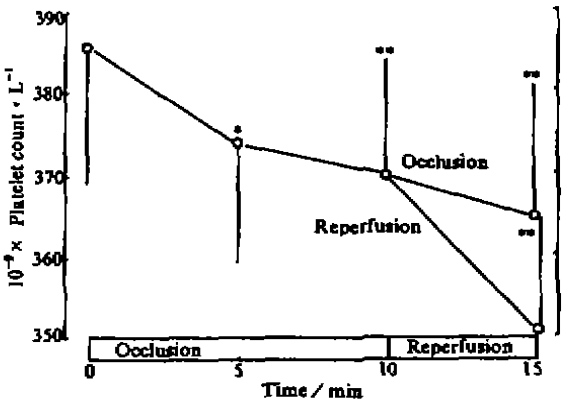

Fig 1. Platelet count during coronary occlusion and reperfusion. $n=10$ rats. $\bar{x} \pm s$. $^aP>0.05$, $^{**}P<0.05$ *vs* normal.

TXB_2 and Keto-$PGF_{1\alpha}$ after reperfusion In saline-treated rats, TXB_2 level was increased and Keto-$PGF_{1\alpha}$ level was remarkably decreased after occlusion and reperfusion ($P<0.01$, Fig 2, Tab 2). TXB_2 levels were decreased, Keto-$PGF_{1\alpha}$ levels and the ratio of Keto-$PGF_{1\alpha}/TXB_2$ elevated obviously in both Que (5 mg·kg^{-1}) and Ver groups *vs* the control group. Que 1 mg·kg^{-1} tended to increase the

Tab 2. Effects of Que on PC, TXB_2, and Keto-$PGF_{1\alpha}$ in blood after reperfusion. $n=7$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs saline.

	Dose/ $mg\cdot kg^{-1}$	PC/ $10^9\cdot L^{-1}$	TXB_2/ $ng\cdot L^{-1}$	Keto-$PGF_{1\alpha}$/ $ng\cdot L^{-1}$	Keto-$PGF_{1\alpha}$ / TXB_2
Saline	—	354±14	1 302±528	51±46	0.04
1% Me_2SO	—	353±13^a	1 309±580^a	53±36^a	0.04^a
Que	0.2	351±14^a	1 226±574^a	58±26^a	0.05^a
	1.0	363±15^a	908±327^a	78±63^a	0.09^a
	5.0	391±12^b	599±195^c	181±83^c	0.30^c
Ver	5.0	392±13^b	507±198^c	177±95^c	0.35^c

Keto-$PGF_{1\alpha}$ level and decrease the level of TXB_2 ($P>0.05$). The level in groups of 0.2 $mg\cdot kg^{-1}$ of Que and 1% Me_2SO were not different from that in the control group.

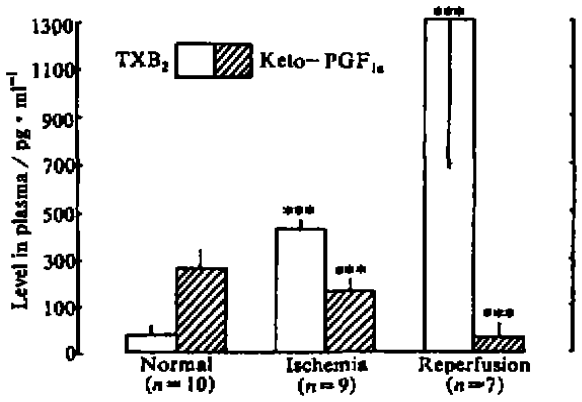

Fig 2. TXB_2 and Keto-$PGF_{1\alpha}$ in rats after coronary occlusion and reperfusion. $\bar{x}\pm s$. $^{***}P<0.01$ vs normal.

Ultrastructure of platelet after reperfusion The platelets after reperfusion lost their discoid contour and became very irregularly with a lot of pseudopods. The cytoplasm of the platelets became coatse and swollen toward the periphery. The open canalicular system was markedly dilated and most of the microtubules were located beneath the plasm membrane. Compared with the control group, Que alleviated the ultrastructural changes of the platelet. No obvious difference in the ultrastructure of platelet in Que 5 $mg\cdot kg^{-1}$ group as compared with that of the normal was seen (Fig 3, Plate 2).

DISCUSSION

The present work confirmed the protective effect of Que on reperfusion arrhythmias with favorable changes of prostanoids and with inhibition of platelet aggregation.

The most severe arrhythmias occured after reperfusion, accompanied by a significant decrease of PC in blood. The reperfusion-induced arrhythmias as well as the decrease in PC were inhibited by Que. The protective actions of Que indicated that arrhythmogenic effect of reperfusion was associated with blood platelet activation.

Intravascular platelet aggregation during reperfusion was manifested in our experiments by a decrease in PC and by deviations in the ultrastructure of platelets. The elevated level of TXB_2 and the Keto-$PGF_{1\alpha}$ level in blood were also evidences that intravascular platelet aggregation occurred during reperfusion. There were some explanations for the relationship between platelets and reperfusion-induced arrhythmias. Platelet aggregation and excretion may be involved in the arrhythmogenic effect of ischemia[5,7]. Blood platelets and active mediators accumulated in the ischemic region seemed to play an important role in the generation of reperfusion arrhyth-

mias. The growth of the ischemic area could be correlated with formation of multiple small thrombi[7,8]. Restoration of blood flow to the ischemic region resulted in displacement of accumulated platelet aggregates towards the microcirculation. The generation of TXB_2 and formation of intravascular platelet aggregation favored a heterogeneous myocardial perfusion, which in turn triggered a chain of events that resulted in the occurrence of severe arrhythmias and even death.

In conclusion, the decrease in PC, the activation of platelets and increase of TXB_2 level in blood were indications of the participation of blood platelets in the arrhythmogenic activity of reperfusion. The reperfusion-induced arrhythmias were inhibited by Que. We proposed that antiarrhythmic action of Que resulted from inhibition of platelet aggregation and TXA_2 formation, the increase of PGI_2 generation and the ratio of PGI_2/TXA_2.

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槲皮素对大鼠血小板和再灌性心律失常的影响

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摘要 本文用在体 Wistar 大鼠心肌缺血再灌模型, 观察血小板与再灌性心律失常的关系及槲皮素的影响. 再灌前2 min iv 槲皮素(5 mg·kg^{-1})可显著降低室颤发生率和 TXB_2值, 明显改善血小板超微结构增加 Keto-$PGF_{1\alpha}$值和血小板数. 结果显示, 槲皮素抗在体大鼠再灌性心律失常作用与抑制血小板聚集、TXA_2形成及增加 PGI_2的产生有关.

关键词 槲皮素; 心律失常; 血小板聚集; 血栓素 A_2; 血栓素 B_2; 依前列醇; 6-酮-前列腺素 $F_{1\alpha}$