An Experimental Myocardial Infarction Model in the Rat and Its Properties

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ABSTRACT—The photochemical reaction between rose bengal and light (540 nm) produces thrombotic occlusion in rat coronary artery. We have now developed an experimental myocardial infarction (MI) model by photochemically induced thrombosis (PIT) in rats and investigated the mechanisms responsible for the induction of MI. PIT in the coronary artery induced myocardial ischemia, which was determined by tissue oxygen tension (tpO_2), and resulted in MI. Pretreatment with a thromboxane (TX) A_2 -receptor antagonist, vapiprost, prevented a decrease in myocardial tpO_2 and markedly reduced the MI area, although vapiprost inhibited collagen-induced platelet aggregation by 30% *ex vivo*. An ADP-induced platelet aggregation inhibitor, clopidogrel, also reduced the MI area. In contrast to vapiprost, clopidogrel inhibited collagen-induced platelet aggregation by 90% *ex vivo*. Pretreatment with a 5-HT₂-receptor antagonist, ketanserin, which did not inhibit collagen-induced platelet aggregation *ex vivo*, prevented the decrease in myocardial tpO_2 and reduced the MI area. These results suggest that TXA₂, 5-HT and ADP play a role in the induction of MI and that platelet aggregation and other factors induce ischemia in this model.

Keywords: Myocardial infarction, Thrombosis (photochemically induced), Thromboxane A_2 , 5-HT, ADP

Development of experimental myocardial infarction (MI) models provide much information concerning human MI. A number of such experimental MI models exist, including occlusion of the coronary artery by ligation (1), insertion of copper coil into an artery (2) and endothelial injury by electrical current (3). The model is selected for use according to the aim of the study; e.g., ischemia-reperfusion, thrombogenesis or arrhythmias. However, an MI model caused by thrombus in rats has not been reported. Thus, mediators involved in the MI model produced by thrombus in rats remain unclear.

Recently, we have reported some photochemically induced thrombosis (PIT) models in rats (4) and guinea pigs (5, 6). In these PIT models, endothelial injury is induced by singlet oxygen generated by a photochemical reaction (7, 8). Fukuchi et al. (9) have previously demonstrated that the photochemically induced thrombus in the coronary artery consisted of platelets, and ventricular arrhythmias were induced. Since their model had high mortality within a few hours, they failed to detect MI in rats. Therefore, we attempted to make an MI model by throm-

bus in the rat coronary artery and investigate representative mediators, thromboxane (TX) A_2 , 5-HT and ADP, involved in MI using antagonists.

MATERIALS AND METHODS

Photochemically induced thrombosis (PIT) in rat coronary artery

Thirty-four male Sprague-Dawley rats (Charles River Japan, Atsugi) weighing about 400 g were anesthetized by i.p. injection of 50 mg/kg of sodium pentobarbital. The jugular vein was cannulated for rose bengal and drug delivery. Tracheal intubation was performed for artificial respiration. The chest was opened by a left thoracotomy. Transillumination with green light (540 nm) was achieved by use of a xenon lamp with a heat absorbing filter and a green filter (Hamamatsu Photonics, Hamamatsu). The head (3 mm in diameter) of the green light source was fixed 5-mm-apart from the surface of the myocardium between the conoventricular vein and branches of the ventricular cordis magna indicated in Fig. 1, since only the vein is visible on the surface of myocardium and the left coronary artery is imbedded in myocardium adjacent to the vein. The distance of 5-mm-apart from the surface

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Fig. 1. The light-irradiated area in the rat heart. a: left auricle, v: left ventricle, closed circle: light-irradiated area.

of the myocardium is necessary to avoid touching the head of the light to the myocardium.

To measure tissue ischemia, an oxygen electrode (0.2 mm in diameter, Inter Medical, Tokyo) was placed in the myocardium (2 mm depth) distally from the head of green light source to monitor myocardial tissue oxygen tension (tpO_2). The oxygen tension at 2 mm depth appeared to show endocardial oxygen tension in the rat heart. After establishing the baseline of the tpO_2 recordings, the green light was switched on and then rose bengal (20 mg/kg) was injected. Ten minutes later, the green light was switched off, and tpO_2 was continuously monitored for a further 50 min. After tpO_2 measurement, the chests of the animals were closed. After rats came out of pentobarbital anesthesia, they were treated with a lubricating surface anesthetic and returned to their cages.

Measurement of MI area

The rat hearts were sectioned into 5 3-mm-thick slices from the apex cordis to the base in planes 24 hr after generating PIT. Four slices (fractions 1–4) from the apex were stained in a solution of 1% 2,3,5-triphenyl tetrazol-

ium chloride (TTC; Tokyo Kasei, Tokyo) in 0.1 M phosphate buffer, pH 7.4. Photos of stained slices were taken on Polaroid film, and MI (white) and non-infarct (red) areas were analyzed with a computer analyzer (Videoplane, Oberkochen, Germany).

Histological study in the model

The rat heart was removed 30 min after the initiation of the photochemical reaction and fixed with 10% phosphate-buffered formaldehyde. The heart was sectioned transversely and stained with hematoxylin-eosin for microscopic observation of the thrombus.

Platelet aggregation in whole blood ex vivo

Twenty-six male rats were used in the experiments. The drugs were injected into the tail veins, and the animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Then arterial blood was collected into tubes containing 3.2% trisodium citrate (9:1, v/v). After the blood was incubated in a Chrono-Log whole blood aggregometer (C550; Chrono-Log, Havertown, PA, USA) for 10 min, the number of unaggregated platelets was deter-

mined by a Hematology Analyzer (MEK-5150; Nihon Kohden, Tokyo). Platelet aggregation was studied in response to collagen (5 μ g/ml; Hormone-Chemie, Munich, Germany).

Drugs

A TXA₂-receptor antagonist, vapiprost (3 mg/kg) (10, 11), and a 5-HT₂-receptor antagonist, ketanserin (1 mg/kg; Sigma, St. Louis, MO, USA), were intravenously injected 10 min before rose bengal injection. Doses of vapiprost and ketanserin used were those reported to prolong the time to femoral arterial occlusion in the rat photochemically-induced thrombosis model (12, 13). An ADP-induced platelet aggregation inhibitor, clopidogrel (20 mg/kg) (14), was intravenously injected 2 hr before rose bengal injection. In studies of platelet aggregation, vapiprost and ketanserin were injected 10 min before blood sampling and clopidogrel was injected 2 hr before sampling.

Statistics

All results are expressed as the mean $\pm$ S.E. All data were analyzed by Dunnett multiple comparison test following analysis of variance. Results were considered to be significantly different if $P < 0.05$.

RESULTS

Induction of MI

In the method reported by Fukuchi et al. (9), the head has been fixed just above the conoventricular vein. Myocardial tpO₂ gradually decreased and plateaued at a

value of 70% at 20 min after the initiation of the photochemical reaction (Fig. 2), whereas that of the sham-operated animals was unaltered (data not shown), and the thrombus generated by the photochemical reaction was seen 30 min after the initiation of the reaction in the irradiated area of the left coronary artery (Fig. 3, A and B). The thrombus appeared to be platelet-rich, similar to that shown by Fukuchi et al. (9). All rats survived 24 hr after generating PIT and five out of eight animals showed a Q-wave type electrocardiogram (Fig. 4). We confirmed that MI was induced from the transillumination area to the apex cordis in TTC-stained sections, and the MI in animals with Q-wave type electrocardiograms showed transmural infarcts (Fig. 4). Sham-operated animals did not show MI.

We investigated which mediators play a role in the induction of MI in this model. Pretreatment with a TXA₂-receptor antagonist, vapiprost (3 mg/kg, i.v.), 10 min before the initiation of photochemical reaction prevented the decrease in tpO₂ during 60 min (Fig. 2) and reduced the MI area at fractions 1 (apex) and 2 (Fig. 5). Pretreatment with a 5-HT₂-receptor antagonist, ketanserin (1 mg/kg, i.v.), reduced the decrease in tpO₂ and also reduced the MI area at fractions 1 (apex), 2 and 3. An ADP-induced platelet aggregation inhibitor, clopidogrel (20 mg/kg, i.v.), did not affect tpO₂ until 10 min after the initiation of the photochemical reaction and then tended to decrease the fall in tpO₂ and reduced the MI area. In a separate experiment, we found that vapiprost, ketanserin and clopidogrel at each dose did not change the heart rate and blood pressure (data not shown).

Fig. 2. Changes in myocardial oxygen tension after initiation of the photochemical reaction. *: $P < 0.05$, **: $P < 0.01$ vs Control. —■—: Control (n=8); —□—: vapiprost, 3 mg/kg (n=8); —●—: ketanserin, 1 mg/kg (n=9); —○—: clopidogrel, 20 mg/kg (n=9).

Fig. 3. Light microscopic observation of photochemically induced thrombosis in the rat coronary artery. A (40 $\times$): A thrombus is seen in the coronary artery (CA). V: Vein. B (400 $\times$): Higher magnification view of the thrombus.

Fig. 4. Typical myocardial infarction and electrocardiogram in photochemically induced thrombosis. The hours indicate time after the initiation of the photochemical reaction. Slices of the heart in the photos are arranged in the following order: fraction 1 (upper-left), fraction 2 (upper-right), fraction 3 (lower-left) and fraction 4 (lower-right).

Fig. 5. Comparison of myocardial infarcted areas of rats treated with drugs. *: $P < 0.05$, **: $P < 0.01$ vs Control. Fractions are numbered from the apex to the base. ■: Control (n=8); ■: vaproprost, 3 mg/kg (n=8); ▨: ketanserin, 1 mg/kg (n=9); ▩: clopidogrel, 20 mg/kg (n=9).

Table 1. Effect of vapirost, ketanserine and clopidogrel on collagen-induced platelet aggregation *ex vivo*

	Control	Vapirost	Ketanserine	Clopidogrel
n	7	7	6	6
Dose (mg/kg)		3	1	20
Aggregation (%)	78.6±2.5	55.5±3.2**	81.6±1.5	8.2±1.5**

**P<0.01 vs Control.

Platelet aggregation in whole blood ex vivo

Since the irradiated rat coronary was occluded by thrombus, we compared effects of the drugs on platelet aggregation induced by collagen, whose exposure at the site of arterial injury appeared to initiate platelet aggregation in the PIT model (4, 9). Pretreatment with vapirost inhibited platelet aggregation by 30% (Table 1). In contrast, clopidogrel inhibited platelet aggregation by 90% and its aggregation was significantly lower than that of vapirost. Ketanserin did not affect platelet aggregation.

DISCUSSION

PIT has been applied to the inner ear microcirculation (15) and femoral artery (4, 6, 16) in animals. In the present paper, we have irradiated the surface of the myocardium between the conoventricular vein and branches of the ventricular cordis magna by the green light, induced thrombosis and MI, and showed that TXA₂, ADP and 5-HT play a role in the induction of MI in rats. In the method reported by Fukuchi et al. (9), the head was fixed just above the conoventricular vein. Since their model showed high mortality within a few hours, it can not be used to evaluate MI. To our knowledge, the present study is the first to describe a MI model caused by thrombus in the rat. This MI model is a useful approach for studying experimental MI and prevention by a drug. In contrast to the MI model produced by ligating the coronary artery, it may be difficult to perfuse our model without fibrinolysis.

The direct continuous measurement of the coronary blood flow in the rat appears to be methodologically much more difficult, since the rat coronary artery is minute in size and completely surrounded by cardiac muscle. Therefore, we tried to measure myocardial tpO₂ as an index of tissue ischemia instead of the coronary blood flow. Sayen et al. (17) have described that myocardial tpO₂ levels fell during occlusion and recovered after release of vessel occlusion in dogs. We previously confirmed that the decreases of myocardial tpO₂ by PIT in the rat coronary artery was comparable to those of tpO₂ by ligation of the artery (data not shown). In the present study,

myocardial ischemia appears to be achieved at 20 min after the initiation of the photochemical reaction (Fig. 2), and the MI area was induced from the illuminated area to the apex. These findings indicate that MI is induced by a deficit in blood flow, not by direct injury by the light.

The TXA₂-receptor antagonist vapirost prevented the decrease in myocardial tpO₂ and reduced MI area most effectively among the tested drugs. This finding suggests that TXA₂/endoperoxides are generated in our model, probably by activated platelets and/or leukocytes. Since vapirost insufficiently inhibited collagen-induced platelet aggregation, only by 30% *ex vivo* and has inhibited vasoconstriction induced by U-46619 (stable TXA₂ analog) *in vitro* (11) and U-46619 and collagen *in vivo* (10), both TXA₂/endoperoxides-induced platelet aggregation and vasoconstriction appear to be related in our model. In the canine model of coronary stenosis associated with endothelial injury, Golino et al. (18) have described that one of the TXA₂ receptor antagonists, SQ29548, abolishes cyclic flow variations and results in an increase in the left anterior descending artery cross-sectional area. It is also known that TXA₂ formation increases in patients with acute MI (19). Therefore, the role of TXA₂ in our MI model is clear and seems similar to the canine model and patients with acute MI.

The 5-HT₂-receptor antagonist ketanserin reduced the MI area and suppressed the decrease in tpO₂. De Clerck (20) has described that 5-HT, which is released by activated platelets at the site of arterial wall injury, contributes to vasoconstriction and platelet-dependent thrombogenesis. In fact, the 5-HT₂-receptor antagonist LY53857 abolished arterial vasoconstriction induced by a constrictor (18). Since ketanserin did not inhibit collagen-induced platelet aggregation in our *ex vivo* study, ketanserin appears to mainly suppress vasoconstriction. However, we can not exclude the possibility that ketanserin inhibits thrombogenesis *in vivo*, since it is known that 5-HT amplifies platelet aggregation induced by ADP, TXA₂, catecholamines and thrombin (20).

A novel ADP-induced platelet aggregation inhibitor, clopidogrel, reduced the MI area and showed marked inhibition of collagen-induced platelet aggregation *ex vivo*. The latter observation appears to indicate that clopidogrel suppressed thrombogenesis caused by the photochemical reaction, since Maffrand et al. (21) have described that ADP plays a key role in thrombogenesis in the rat, and another ADP-induced platelet aggregation inhibitor, ticlopidine, suppressed PIT in the rat femoral artery (12).

In conclusion, TXA₂, 5-HT and ADP appear to play a role in this MI model and these mediators appear to induce platelet aggregation or other factors involved in the ischemic injury.

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