Infarct Size and the Protection of Ischemic Myocardium in Pig, Dog and Human

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To define whether recanalization after occlusion can reduce the myocardial infarct size, we compared the infarct size in 25 pig hearts without collateral circulation, 35 dog hearts with collateral circulation and 11 human autopsied hearts with coronary thrombolysis at 2 to 6 hours after the onset of acute myocardial infarction.

The data showed that % infarct size in the risk area increased according to the duration of occlusion. In the pig, % infarct size was $80 \pm 9\%$ in the recanalization after 1 hour occlusion and $96 \pm 2\%$ in the recanalization after 2 hour occlusion. There was no significant difference between these and the permanent occlusion group ($95 \pm 3\%$). In the dog, % infarct size was $35 \pm 31\%$ in the recanalization after 4 hour occlusion and $59 \pm 27\%$ in the permanent occlusion group. In human autopsied hearts, the infarct size was the same between the recanalization group ($82 \pm 6\%$) and the permanent occlusion group ($80 \pm 11\%$). The % infarct size in the recanalization groups was less than or the same as that in the hearts with permanent occlusion in dog, pig and human. Thus, it is concluded that, to reduce conclusively the infarct size, recanalization should be done within 1 hour after the occlusion in the hearts without collateral circulation and within 4 hours in the hearts with collateral circulation. So called reperfusion injury which means the greater expansion of the % infarct size than that in the permanent occlusion is not present.

SINCE Rentrop et al first described selective intracoronary thrombolysis in patients with acute myocardial infarction (AMI) in 1979,¹ coronary thrombolysis and/or percutaneous transluminal coronary angioplasty have been used more and more widely.^{2,3} An increased rate

of recanalization in the infarct-related coronary artery and a lower death rate at the acute stage have been established. However, there are controversies about whether these procedures can salvage the ischemic myocardium and reduce infarct size in human AMI²⁻⁵ To define whether recanalization after occlusion can reduce the infarct size, we compared the infarct size in the pig hearts without collateral circulation^{6,7} the dog hearts with collateral circulation⁸ and human autopsied hearts.

Key words:

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Fig.1. Histology of contraction band necrosis (left) and coagulation necrosis (right) (Masson's trichrome stain $\times 200$)

MATERIAL AND METHODS

Experimental study

Twenty five farm pigs weighing 25 to 43 kg each were used. These pigs were divided into 5 groups of five pigs each according to the duration of occlusion of the left anterior descending coronary artery (20, 30, 60, and 120 min of occlusion in groups 1, 2, 3, and 4, respectively). The heart of each pig in these 4 groups was reperfused for 8 hours. The pig hearts in group 5 were not reperfused. In the dogs, 35 mongrel dogs were divided into 3 groups, according to the duration of occlusion; 10 dogs with 2-hour occlusion followed by 9-hour recanalization (group 1); 10 dogs with 4-hour occlusion followed by 9-hour recanalization (group 2); 15 dogs with 12-hour occlusion (permanent occlusion: group 3). The left anterior descending coronary artery was completely occluded with a Vesseloops (Med General) rubber band. Reperfusion was then done for 8 hours. Regional myocardial blood flow was measured before occlusion and at 30-min intervals after occlusion by the generated hydrogen gas clearance method. The risk area was determined by the postmortem angiography method.⁹ The heart was sliced into 1 cm serial sections in a plane parallel to the atrioventricular groove and x-ray photographs of all slices were taken. The risk area was clearly demarcated by the absence of

contrast medium. These slices were then fixed with 10% formalin, embedded in paraffin, cut into slices 4 μ m thick, and stained with hematoxylin and eosin and Masson's trichrome.

Microscopically, the infarct area was examined for areas of coagulation necrosis and those of contraction band necrosis (Fig. 1). Quantification was done by the method of Fujiwara et al.¹⁰

Human autopsied study

The 11 autopsied patients without old myocardial infarction in the risk area, in whom thrombolysis was performed 2 to 6 hours after the onset of AMI and in whom the time between the onset of AMI and death was 7 hours to 7 days, were analyzed in this study. The clinical diagnosis of AMI was based on severe chest pain lasting more than 30 min, serial electrocardiographic findings of ST-T changes and/or the appearance of abnormal Q waves, and an increase in creatine kinase (CK), SGOT, and lactate dehydrogenase (LDH) levels. In six patients, the infarct-related coronary artery was totally occluded before thrombolysis and was successfully recanalized (group I). Five patients had total occlusion before and after thrombolysis and at autopsy (group II). There were no significant differences between the 2 groups in age, time from the onset of AMI to thrombolysis, time from the onset to death, the cause of death, or the

TABLE I MYOCARDIAL REGIONAL BLOOD FLOW IN THE RISK AREA AFTER OCCLUSION OF CORONARY ARTERY IN PIG AND DOG

Duration of occlusion		Before	30 minutes	3 hours	12 hours
Pig	end	100%	2 ± 2%*	2 ± 2%*	3 ± 2%*
	epi	100%	3 ± 2%	2 ± 2%	2 ± 2%*
Dog	end	100%	12 ± 9%*	21 ± 14%*	25 ± 11%*
	epi	100%	14 ± 8%*	30 ± 12%*	39 ± 19%*

*: $p < 0.05$ to the data before occlusion

TABLE II INFARCT SIZE IN THE RISK AREA AFTER OCCLUSION OF CORONARY ARTERY IN PIG, DOG AND HUMAN

Percent infarct size in the risk area						
	Duration of occlusion until recanalization					Permanent occlusion
	20 min	30 min	1 hr	2 hrs	4 hrs	
Pig	0%	11 ± 7%*	80 ± 9%	96 ± 2%	95 ± 3%	
Dog	0%			24 ± 23%*	39 ± 30%	63 ± 27%
Human					82 ± 6%	80 ± 11%

*: $p < 0.05$ to permanent occlusion group.

TABLE III THE AREA WITH CONTRACTION BAND NECROSIS IN THE INFARCT AREA

Percent CBN in the infarct area					
	Duration of occlusion until recanalization				Permanent occlusion
	30 min	1 hr	2 hrs	4 hrs	
Pig	100 ± 0%	68 ± 11%	2 ± 1%		2 ± 2%
Dog			98 ± 3%	86 ± 12%	55 ± 29%
Human				20 ± 9%	3 ± 3%

degree of collateral circulation. Urokinase from 720,000 to 1,200,000U was used in these patients.

Postmortem coronary angiography was done by the methods of Lee et al. and Koyanagi et al. and the risk area was determined by carefully following the course of each vessel. Four whole transverse thin slices from the upper, middle, lower middle, and lowest portions of the heart were divided into six to eight sections each, which were embedded in paraffin, cut into 4 μ m thick slices, and stained with hematoxylin-eosin and Masson's trichrome. Microscopically, the infarct area was classified as coagulation necrosis or contraction band necrosis (Fig. 1).

RESULTS

Regional blood flow (Table I)

During occlusion of the left anterior descending coronary artery, both endocardial and epicardial regional blood flow were reduced to less than 5% of the control before occlusion in each of the pigs. After reperfusion, the regional blood flow returned to the control level in groups 1 and 2 of the pigs. However, in groups 3 and 4 of the pigs the regional blood flow was less than that before occlusion. The decrease was more prominent in group 4 (Epi/End = 50 ± 13/44 ± 15) than in group 3 (78 ± 6/74 ± 8%).

In group 3 of the dogs, the regional blood flow of the subepicardium and subendocardium

Fig.2. The relationship among reversible and irreversible cellular damage, contraction band necrosis, coagulation necrosis and bleeding during ischemia and reperfusion.¹⁴ Depending on the duration of ischemia, the degree of ischemic myocardial cellular damage increases and finally progresses to coagulation necrosis. The degree is classified into reversible, early irreversible and late irreversible cellular damage. No necrosis is observed when reperfusion is performed at the stage of reversible cellular damage. However, at the stage of early irreversible cellular damage, reperfusion is associated with contraction band necrosis, and at the stage of late irreversible cellular damage with coagulation necrosis and bleeding. Reperfusion rapidly accelerates myocardial cell necrosis. Note that acute curve of reperfusion indicates acceleration of cell necrosis in this figure. If it is possible to reduce the infarct areas by controlled reperfusion, the target tissue is myocytes with early irreversible cellular damage. However, it is unknown whether controlled reperfusion can salvage the myocytes with early irreversible cellular damage. CBN: contraction band necrosis, CN: coagulation necrosis, B: bleeding, $\Rightarrow$: reperfusion

decreased markedly at 30 min after occlusion ($14 \pm 8\%/12 \pm 9\%$) and was relatively restored from 180 min ($33 \pm 12\%/21 \pm 14\%$) to 12 hours ($39 \pm 19\%/25 \pm 11\%$) in spite of complete occlusion of coronary artery. In group 1 of the dogs the regional blood flow returned to the control value completely. However, in group 2 of the dogs, the subendocardial and subepicardial regional blood flows were $72 \pm 32\%$ and $72 \pm 20\%$ of the control values, respectively.

Infarct in the risk area (Table II)

In the pigs, the percent infarct area after reperfusion increased significantly with the duration of occlusion: $0 \pm 0\%$, $11 \pm 7\%$, $80 \pm 9\%$, and $96 \pm 2\%$ in groups 1, 2, 3, and 4, respectively. The percent infarct area was similar in groups 4 and 5 ($95 \pm 3\%$ in group 5).

In the dogs, the percent infarct area in the risk area increased significantly according to the duration of occlusion; $24 \pm 23\%$ in group 1, $39 \pm 30\%$ in group 2 and $63 \pm 27\%$ in group 3.

In the humans, the infarct area was $82 \pm 6\%$ of the risk area in group 2, and $80 \pm 11\%$ in group 2. There were no statistical differences

between the two groups.

The percent areas of contraction band and coagulation necrosis in the infarct area (Table III)

In the pigs, the infarct area was examined to identify areas of contraction band necrosis and those of coagulation necrosis. The percent contraction band necrosis was $100 \pm 0\%$ in group 2, $68 \pm 11\%$ in group 3, and $2 \pm 1\%$ in group 4, and it decreased with the duration of occlusion (Fig. 2). The percent contraction band necrosis in group 4 was similar to that seen in pigs with permanent occlusion (group 5, $2 \pm 2\%$). In group 2, there was little evidence of coagulation necrosis and the infarct area almost always showed contraction band necrosis.

In the dogs, the % area of contraction band necrosis in the infarct area was $98 \pm 3\%$ in group 1, $86 \pm 12\%$ in group 2 and $55 \pm 29\%$ in group 3 ($p < 0.05$, group 1 vs 3, group 2 vs 3).

In the humans, the area of contraction band necrosis was $20 \pm 9\%$ (13.2% to 37.5%) of the infarct area (contraction band necrosis plus coagulation necrosis) in group 1 and $3 \pm 3\%$ (0 to 5.8%) in group 2. This figure was significantly

higher in group 1 than in group 2.

DISCUSSION

The present data revealed that % infarct size in the risk area in the hearts with recanalization after occlusion of the coronary artery was less than or the same as that in the hearts with permanent occlusion in dogs, pigs and humans. In the permanent occlusion group, % infarct size in the risk area was smaller in the dog with rich collateral circulation ($59 \pm 27\%$) than in the pig without collateral circulation ($95 \pm 3\%$). These indicate that so called reperfusion injury^{7,11,12} which means the greater expansion of the % infarct size than that in the permanent occlusion is not present. The presence of rich collateral circulation can also reduce the infarct size even in the permanent occlusion.

The present study revealed that, in the dog heart with collateral circulation, diffuse contraction band necrosis was seen even in the permanent occlusion group. Contraction band necrosis is generally considered an indicator of reperfusion to the myocyte with early irreversible cellular damage, and coagulation necrosis with hemorrhage an indicator of reperfusion to myocytes with late irreversible cellular damage (Fig 2). The present study confirmed the findings that the regional myocardial blood flow through collateral circulation increases during occlusion of the coronary artery.¹³ Thus, diffuse contraction band necrosis in the permanent occlusion group of dog depends on reperfusion in the risk area via collateral vessels.

Coronary thrombolysis and percutaneous transluminal coronary angioplasty are currently the most important treatment for acute myocardial infarction.¹⁻³ However, results of double-blind trials are conflicting with respect to whether or not coronary thrombolysis can limit the infarct area. The present data showed that, in the pig hearts without collateral circulation, % infarct size in the risk area was 80% even in the recanalization after 1 hour occlusion. In the dog hearts with collateral circulation, there was no significant difference of % infarct size between the recanalization group after 2 to 6 hours occlusion and the permanent occlusion group. In human autopsied hearts, in which coronary thrombolysis was done 2-6 hours after the onset of acute myocardial infarction, infarct size in the risk area was the same between the recanalization group and the permanent occlu-

sion group.

Thus, we conclude that, to reduce definitively the infarct size, recanalization should be done within 1 hour after the occlusion in the hearts without collateral circulation and within 4 hours in the hearts with collateral circulation.

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