Limitation of Coronary Thrombolysis:
Experimental Study of Reperfusion After Ischemia

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Contraction band necrosis (CBN), coagulation necrosis (CN) and infarct size, expressed as CBN + CN, were quantitatively analyzed in 15 pig hearts without collateral circulation. The left anterior descending coronary artery was ligated for 60 and 120 minutes and then reperfused for eight hours (group 1 and 2, respectively). Five hearts were not reperfused (group 3). There was no change in hemodynamics with either occlusion or reperfusion. Regional myocardial blood flow, measured by the generated hydrogen gas clearance method, decreased to almost zero after occlusion but recovered during reperfusion. Percent infarct area of the perfused area in group 1, 2 and 3 were 80 ± 9, 96 ± 2% and 95 ± 3%, respectively. The percent area of CBN was 68 ± 11% in group 1, 2 ± 1% in group 2 and 2 ± 2% in group 3. We conclude that in pig hearts without collateral circulation, the transmural infarct, two-thirds of which is occupied by CBN, is evident even in reperfusion following one-hour occlusion. Therefore, in patients with acute myocardial infarction, coronary thrombolysis should be performed within one hour after the onset of the infarction to reduce the infarct size.

Reperfusion after ischemia by coronary thrombolysis and angioplasty are current treatment for acute myocardial infarction. An increased rate of recanalization of the infarct-related coronary artery and a lower death rate at the acute stage have been reported. However, it has not been established whether coronary thrombolysis can reduce the size of the infarct. Reperfusion at the early stage of ischemia is known to cause contraction band necrosis, and at the late stage of ischemia, coagulation necrosis and hemorrhage occur. These studies were done using dog models with rich collateral circulation. Few studies have been done on the histology in an animal model without collateral circulation. Therefore, we quantitatively determined the extent of infarct size, contraction band necrosis and coagulation necrosis in the pig hearts without collateral circulation in reperfusion after ischemia.

METHODS

Fifteen farm pigs each weighing 25–43 kg were used. These pigs were divided into three groups of five pigs each, according to the duration of occlusion of left anterior descending coronary artery. In groups 1 and 2, the artery was occluded for 60 and 120 minutes respectively. Then, the artery was reperfused for eight hours. The pig hearts in group 3 were subjected to eight hour occlusion and were not reperfused.

The pigs were sedated with ketamine (2 mg/kg), and anesthetized with sodium with ketamine (20 mg/kg).

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Fig. 1. Progression of the infarct area in the pig heart according to the duration of occlusion in preparation stained with nitrotetrazolium.

Upper: reperfusion after 60 minute occlusion, middle: reperfusion after 120 minute occlusion, lower: permanent occlusion. Dot lines indicate perfused area of the infarct related artery. Infarct area is not stained with nitrotetrazolium blue.

Through a median sternotomy, the heart was exposed and suspended in a pericardial cradle. A 5-mm segment of the distal one-third of the left anterior descending coronary artery was dissected free from the surrounding tissue. After obtaining the base-line measurement of the aortic pressure (Statham P 23Db), the limb lead of ECG, and epicardial and endocardial regional blood flow in the perfused area by the generated hydrogen gas clearance method, the left anterior descending coronary artery was completely occluded with a Vesselloops (Med General) rubber band. Then, it was reperfused for eight hours as described above. After sacrifice, postmortem coronary arteriography was performed to determine the perfused area supplied by the occluded artery. Next, the heart was sliced into 1 cm serial sections, in a plane parallel to the atroventricular groove and X-ray photographs of all slices were taken. The perfused area was clearly demarcated by the absence of contrast medium. All slices including the ischemic area were incubated for 15–30 minutes at 37°C in 0.01 M phosphate buffer solution at pH 7.4 containing nitrotetrazolium blue (NTB, Sigma: 50 mg/100 ml). The infarct area was identified macroscopically by the absence of a dark blue color (Fig. 1). These slices were then fixed with 10% formalin, embedded in paraffin, cut into slices 4 μ thick, and stained with hematoxylin and eosin, and also Masson's trichrome.

Microscopically, the infarct area was classified into coagulation necrosis and contraction band necrosis (Fig. 2). The infarct area observed microscopically was almost the same as the macroscopic infarct area, estimated after NTB staining, before fixation in each heart. Therefore, we used the area of infarct, determined by microscopic analysis.

Quantification was done by our previously reported method? The infarct area was expressed as a percentage of the perfused area of the occluded artery, and the areas of contraction band necrosis as a percentage of the infarct area. The values were expressed as round numbers, with fractions of 0.5 or more counted as 1 and those less than 0.5 not counted.

Statistical analysis

Statistical analysis was performed using repeated measure analysis for hemodynamic and blood flow data. The histologically determined area was analyzed by a one way analysis of variance and the multiple comparison test. The level of significance was taken as p < 0.05.

RESULTS

Heart rate and aortic pressure showed no significant changes before, during occlusion or after reperfusion.

Before occlusion of the left anterior descending coronary artery, the mean endocardial and epicardial blood flow was 90 ± 16 and 92 ±
Fig. 2. Contraction band necrosis (left) and coagulation necrosis (right).
Note that hemorrhage is seen in the tissue area with coagulation necrosis (Masson trichrome stain, × 200).

Fig. 3. Regional myocardial blood flow in the pig heart. Regional blood flow in the inner (I) and outer (O) thirds of the left ventricular wall measured by the hydrogen gas clearance method is almost zero occlusion of the coronary artery and recovers after reperfusion.

17 ml/min/100g in the 15 pigs, respectively; with no significant difference (Fig. 3). During occlusion, both endocardial and epicardial blood flow was reduced to less than 6 ml/min/100g, in each heart. After reperfusion, the regional blood flow returned to 52 ± 16 ml/min/100g in group 1 and 47 ± 18 ml/min/100g in group 2.

The percent infarct area was 80 ± 9%, 96 ± 2% and 95 ± 3% in groups 1, 2 and 3. The infarct area was classified into contraction band necrosis and coagulation necrosis. The percent contraction band necrosis was 68 ± 11% in group 1, 2 ± 1% in group 2 and 2 ± 2% in group 3. In group 1, the percent contraction band necrosis was 26 ± 12% in the inner third, 84 ± 20% in the middle third and 90 ± 13% in the outer third (inner vs. outer and middle; p < 0.05) (Fig. 4). Most of the necrosis was coagulation necrosis in the inner third and contraction band necrosis in the middle and outer thirds. The percent contraction band necrosis in the inner, middle and outer thirds was 0 ± 0, 1 ± 1 and 2 ± 2% in group 2 respectively, and 1 ± 1, 1 ± 1 and 2 ± 3% in group 3, respectively. In groups 2 and 3, contraction band necrosis was found only at the edge of the area of infarction, especially beneath the epicardium.

In group 1, congestion of blood cells in the
microvessels and slight hemorrhage were present in the infarct area, especially in tissues with coagulation necrosis. In group 2, moderate to severe hemorrhage was diffusely seen in the infarct area. Diffuse hemorrhage was evident at the core of the infarct. There was no evidence of definite hemorrhage in group 3.

**DISCUSSION**

The dynamic relationships between duration of occlusion, and infarct area, contraction band necrosis, coagulation necrosis or hemorrhage are shown schematically in Fig. 4.

The percent infarct area increased with duration of the occlusion and the level after 120 minutes of occlusion was the same as that seen in cases of permanent occlusion. In previous studies done to determine whether reperfusion can salvage the ischemic myocardium of dogs, the percent infarct area to the area at risk was 28, 70, 72 and 79% with 40 minutes, 3 hours, 6 hours occlusion and permanent occlusion, respectively. In pigs, the percent infarct area was 80% and transmural infarction appeared even in cases of reperfusion after 60 minutes of occlusion. This difference can be explained by the lack of collateral flow in the pig hearts.

The present study revealed that 1) coagulation necrosis occurred transmurally in ischemic hearts not reperfused; 2) with early reperfusion after 60 minute occlusion, contraction band necrosis occurred diffusely, and with late reperfusion after 120 minute occlusion, coagulation necrosis occurred. With reperfusion after 60 minutes of occlusion, the inner third of the ischemic wall showed coagulation necrosis and the middle and outer third contraction band necrosis. These findings indicate that ischemic cellular damage immediately before reperfusion is milder in myocytes with contraction band necrosis than in those with coagulation necrosis. However, myocytes in which the contraction band necrosis appears after reperfusion are generally considered to be irreversibly damaged immediately before reperfusion. However, several studies suggest that protection against contraction band formation may enable the cells to survive an otherwise fatal insult. Since the contraction band formation of the myofibril is related to calcium overload in cases of reperfusion, the prevention of calcium overload using calcium-free cardioplegic solution and calcium blockers should be considered.

Hemorrhage was slight in group 1, marked in group 2, but rare in group 3. Hemorrhage was almost always localized within the boundaries of the infarct. This confirms our previous findings in acute myocardial infarction patients with coronary thrombolysis. Microvascular cellular damage in the presence of ischemia slowly follows damage of myocytes. Thus, hemorrhage
occurs in tissues already markedly damaged and that it rarely extends the infarct zone.

We conclude that, in pig hearts without collateral circulation, the transmural infarct is evident even in reperfusion following one hour occlusion. Therefore, in patients with acute myocardial infarction, coronary thrombolysis should be performed within one hour after the onset of infarction.

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