The Experimental Study of the Coronary Reperfusion in the Acute Myocardial Ischemia: The Feasibility of the Myocardial Salvage

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In order to know the feasibility of coronary reperfusion by thrombolysis or aorto-coronary bypass graft in the early stages of the acute myocardial infarction, we studied the effect of the coronary artery reperfusion to acutely ischemic myocardium induced by the coronary artery occlusion in ninety-five anesthetized open-chest dogs. The major factors determining the extent of the myocardial salvage by the reperfusion were the duration of the occlusion time and the degree of the reperfusion injury. These two determinants were analysed by coronary circulation, the regional myocardial function, the mitochondrial metabolism, mitochondrial Ca and Mg contents, and morphological findings of the myocardium by electron-microscopy. The regional myocardial contractility (% systolic shortening) and the mitochondrial metabolism (oxydative phosphorylation) were significantly damaged by the reperfusion more in 60 minute occlusion than in 30 minute occlusion, although the coronary circulation (coronary blood flow, regional myocardial blood flow and coronary vascular resistance) and myocardial gas contents (PO₂, PCO₂ and pH) in the ischemic myocardium induced by less than 60 minute occlusion were almost recovered to the pre-occluded level by 60 minutes after reperfusion. By 120 minute reperfusion, the ischemic damage calculated from mitochondrial Ca and Mg contents (MC index: 1-[Mg/Ca] ischemia/[Mg/Ca] non-ischemia) was not changed in 30 minute occlusion but was significantly deteriorated in 60 minute occlusion. Therefore, coronary reperfusion must be started within 60 minutes or less after occlusion. A supplementary way to protect the myocardium from ischemia is needed as soon as possible before reperfusion. Ca\textsuperscript{2+} antagonist, diltiazem, administered 10 μg/kg/min intravenously during the coronary occlusion and reperfusion significantly improved the myocardial contractility, the mitochondrial metabolism, MC index and morphological findings by electron-microscopy in reperfusion after 60 minute occlusion. Therefore, it is useful to administer Ca\textsuperscript{2+} antagonist, diltiazem, to protect the myocardium from ischemia until thrombolysis or aorto-coronary bypass graft is successful.

ALTHOUGH thrombolysis\textsuperscript{1} and aorto-coronary bypass graft\textsuperscript{2} have recently been used to treat the early stages of acute myocardial infarction, these treatments have not been shown to clearly protect the myocardium from ischemia. It appears that the earlier these treatments could be started, the more effective they are for the patients\textsuperscript{3} Therefore, it is essential to know exactly when to start these treatments in order to salvage the ischemic myocardium. The major determinants in salvaging the ischemic myocardium in the acute myocardial infarction are the duration of the ischemia\textsuperscript{4} and the degree of the reperfusion injury\textsuperscript{5} In this study, we will try
to discover the time limit during which the ischemic myocardium can be saved by the reperfusion and the reperfusion injury. Also, we will study the possibility of using Ca\(^{++}\) antagonists to salvage the ischemic and reperfused myocardium.

METHODS

Ninety-five mongrel dogs of either sex weighing from 16 to 27 kg were anesthetized with intravenous sodium pentobarbital (25 mg/kg) and maintained with supplemental doses. Artificial respiration with room air was instituted with a Harvard respirator. After thoracotomy through the fifth left intercostal space, the left anterior descending coronary artery (LAD) was dissected between the first and second diagonal branch and fitted with a snare to produce the ischemic and reperfused myocardium. In order to study the regional myocardial function, a pair of sonocrystals was implanted in the subendocardial and subepicardial layers of the left ventricle and oriented parallel to the minor axis. The segmental length of end-diastole and end-systole (EDL and ESL) was measured with a pulse transit sonomicrometer (Ultrasonic Demension System Model 401, Schuessler and Associates, USA). The regional myocardial contractility was manifested as % systolic shortening (%SS) calculated from the segmental length (%SS: [EDL-ESL]/[EDL] × 100). To measure the regional myocardial blood flow (MBF), thermocouples (Model 6L01-S, San-ei, Japan) were implanted in the subendocardial and subepicardial layers. In the middle layer of the ischemic and reperfused myocardium, the myocardial tissue PO\(_2\), CO\(_2\) and pH were measured by the sensors of PO\(_2\), CO\(_2\) and pH. LAD flow was measured by the electromagnetic flowmeter, and aortic and left ventricular pressures were measured by the catheter-tip manometers. To study the mitochondrial function of the myocardium, mitochondria were extracted from the myocardium by Chance and Higihara's method\(^6\). The extracted mitochondria were measured for the oxidative phosphorylation by Clark type O\(_2\) electrode and Ca and Mg contents by Sparrow and Johnstone's method\(^7\) to calculate MC index (1–[Mg/Ca] ischemia/...
[Mg/Ca] non-ischemia). In order to investigate the effect of diltiazem to the ischemic and reperfused myocardium, diltiazem was intravenously infused 10 μg/kg/min from 10 minutes before LAD occlusion to the end of the experiment. After the experiment, the ischemic and reperfused myocardium was morphologically investigated by the electron-microscope.

**PROTOCOL**

The protocol was shown in the Fig. 1.

First: In 31 dogs, LAD was occluded for 30 minutes (6 dogs) and for 60 minutes (6 dogs), and reperfused for 120 minutes after 30 minute occlusion (6 dogs) and 60 minute occlusion (6 dogs). These four groups were compared to the control group (7 dogs). The measuring parameters were as follows: %SS, PO₂, PCO₂ and pH as the regional myocardial function; LAD flow, MBF and coronary vascular resistance (CVR) as the coronary circulation; electron-microscopic findings as the morphological study.

Second: 40 dogs were divided into 6 groups which were the control (8 dogs), 30 minute occlusion (7 dogs), 60 minute occlusion (7 dogs), 180 minute occlusion (6 dogs), and 120 minute reperfusion after 30 minute occlusion (6 dogs) and 60 minute occlusion (6 dogs). In these groups, State 3 and Ca, Mg contents were measured in the ischemic and reperfused myocardium.

Third: In 24 dogs, the effect of Ca⁺⁺ antagonist, diltiazem, on the regional myocardial function (EDL, ESL, %SS, PCO₂, and pH), the mitochondrial function (State 3 and Ca, Mg contents) and morphological findings (electron-microscopic study) was studied.

**STATISTICAL ANALYSIS**

Data were presented as mean ± SEM. Student’s t test was used and differences were considered significant when p <0.05.

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Fig. 3. The regional mechanical function of the myocardium: the change of the segmental length before and during LAD occlusion and after reperfusion.

RESULTS

In Fig. 2, coronary circulation was shown in the group of 120 minute reperfusion after 30 or 60 minute occlusion. LAD flow and MBF of the ischemic myocardium showed the deferred hyperemic response observed in the reperfusion after the short-time occlusion of the coronary artery. LAD flow recovered to the pre-occluded level 10 minutes after reperfusion and MBF recovered 60 minutes after. CVR also recovered to the pre-occluded level 60 minutes after reperfusion in 30 minute occlusion and 90 minutes after reperfusion in 60 minute occlusion, and was continued till 120 minutes after reperfusion. CVR tended to be always lower in the subendocardial layer than in the subepicardial layer. The tissue PO₂, PCO₂ and pH in the ischemic myocardium recovered about 60 minutes after reperfusion in the occlusion for 30 or 60 minutes.

In Fig. 3, the regional mechanical function of the ischemic myocardium was shown by the segmental length in the groups of 120 minute reperfusion after 30 or 60 minute occlusion. EDL and ESL in the ischemic myocardium lengthened just after occlusion and showed systolic bulging. %SS rapidly decreased to the negative level and became stable 30 minutes after occlusion. EDL, ESL and %SS recovered after reperfusion. EDL and %SS after reperfusion in 30 minute occlusion were significantly different from those in 60 minute occlusion (*: p < 0.05).

Mitochondrial function was observed in the second 6 groups shown in Fig. 4. State 3 in the oxydative phosphorylation of the ischemic mitochondria was gradually depressed as the oc-
Fig. 4. Oxydative phosphorylation and Ca, Mg content of mitochondria.

cclusion time was longer until 60 minutes. But State 3 of 180 minute occlusion was almost the same as that of 60 minute occlusion. After reperfusion, State 3 almost recovered to the pre-occluded level in 30 minute occlusion but was more depressed than in 60 minute occlusion (p < 0.05). In the mitochondria of the ischemic myocardium, Ca content was gradually increased and Mg content was gradually decreased till 60 minute occlusion, but these contents in 180 minute occlusion were the same as in 60 minute occlusion. After reperfusion, these contents in 30 minute occlusion were not changed, while Ca content was strongly increased and Mg content was significantly decreased in 60 minute occlusion. With diltiazem treatment, State 3 was not so depressed in 60 minute occlusion and in 120 minute reperfusion after 60 minute occlusion compared to the same occlusion time without treatment. The increase of Ca content after reperfusion was strongly depressed by diltiazem (p < 0.05). The decrease of Mg content after reperfusion was also tended to be depressed by this drug.

Compared to 0.06 ± 0.05 in the control, MC index was increased to 0.18 ± 0.05 in 30 minute occlusion and to 0.10 ± 0.04 in 120 minute reperfusion after 30 minute occlusion.
It was significantly increased to 0.37 ± 0.02 in 60 minute occlusion (p < 0.01) and 0.58 ± 0.09 in 120 minute reperfusion after 60 minute occlusion (p < 0.01). In 60 minute occlusion, it was significantly decreased to 0.19 ± 0.04 by diltiazem (p < 0.01). In 120 minute reperfusion after 60 minute occlusion, it was significantly decreased to 0.29 ± 0.07 by this drug (p < 0.05).

In Fig. 5, the effect of diltiazem on regional mechanical function of the ischemic myocardium was studied by observing EDL, ESL and %SS. Although these parameters in the occlusion were not significantly different between treated and non-treated occlusions, these parameters after reperfusion resembled those of 30 minute occlusion with the non-treatment.

The ischemic injury of the myocardium in these 8 groups was electron-microscopically observed, as shown in Fig. 6.

In 30 minute occlusion, nuclei and mitochondria were slightly injured. In 60 minute occlusion, nuclei were injured with almost complete clearing of the nucleoplasm and mitochondria were injured with more pronounced clearing of the matrix and fragmentation of the Cristae. Nuclei and mitochondria were irreversibly injured in 180 minute occlusion. Nuclei and mitochondria were almost normal in 120 minute reperfusion after 30 minute occlusion, but these were irreversibly injured with clumped chromatin and electron dense matrix in 120 minute reperfusion after 60 minute occlusion. These electron-microscopic findings were slightly reduced by diltiazem.

DISCUSSION

In order to know the feasibility of coronary reperfusion by thrombolysis¹ or aorto-coronary bypass graft² in the early stages of the acute myocardial infarction, we studied the effect of the coronary artery reperfusion on the acutely...
Fig. 6. Electron-microscopic findings in the occlusion and reperfusion

30 minute occlusion: slightly injured nucleus showing some clearing of the nucleoplasm with marginally clumped chromatin; slightly injured mitochondria showing disappearance of the matrix granules and some clearing of the matrix.

60 minute occlusion: injured nucleus with almost complete clearing of the nucleoplasm; injured mitochondria with more pronounced clearing of the matrix and fragmentation of cristae.

180 minute occlusion: irreversibly injured nucleus with complete clearing of the nucleoplasm; irreversibly injured mitochondria with electron dense matrix.

30 minute occlusion and 120 minute reperfusion: almost normal nucleus and mitochondria.

60 minute occlusion and 120 minute reperfusion: irreversibly injured nucleus and clumped chromatin; irreversibly injured mitochondria with electron dense matrix. contraction band (+)

60 minute occlusion with diltiazem: severely injured mitochondria without electron dense matrix.

60 minute occlusion and 120 minute reperfusion with diltiazem: irreversibly injured nucleus and clumped chromatin; irreversibly injured mitochondria with electron dense matrix. contraction band (+)

Ischemic myocardium induced by the coronary artery occlusion in 95 anesthetized open-chest dogs. The major factors determining the extent of the myocardial salvage by reperfusion were the duration of the occlusion time and the degree of reperfusion injury. Therefore, we analyzed these two determinants by the coronary circulation, the regional myocardial function, the mitochondrial metabolism, mitochondrial Ca and Mg contents, and morphological findings of the ischemic and reperfused myocardium by electron-microscopy.

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In 30 or 60 minute occlusion, LAD flow, MBF and CVR in the ischemic myocardium were almost recovered to the pre-occluded level 60 minutes after reperfusion. The myocardial tissue PO$_2$, PCO$_2$ and pH were also recovered to the pre-occluded level 60 minutes after reperfusion. Almost all of the ischemic myocardium appeared to be almost all recovered within 60 minutes after reperfusion. But, the regional mechanical function, especially EDL and %SS, was not recovered to the preoccluded level in spite of reperfusion. In 60 minute occlusion, the regional mechanical function was severely injured after reperfusion. The mitochondrial function was also injured. State 3 in the oxidative phosphorylation of the ischemic mitochondria was gradually depressed as the occlusion time was longer till 60 minutes. After reperfusion, State 3 was almost recovered to the pre-occluded level in 30 minute occlusion but was more depressed in 60 minute occlusion. In 180 minute occlusion, State 3 and the regional mechanical function were almost the same as those in 60 minute occlusion. In mitochondria, Ca content was gradually increased and Mg content was gradually decreased till 60 minutes after occlusion, but these contents in 180 minute occlusion were the same as in 60 minute occlusion.

Therefore, it is possible that the ischemic myocardium can be salvaged to some extent if the reperfusion is started within 60 minutes after occlusion. The earlier the reperfusion can be started, the more effective to the ischemic myocardium it will be. In 30 minute occlusion, the regional mechanical function of the ischemic myocardium was still depressed after reperfusion, but the mitochondrial metabolism and Ca, Mg contents were exceedingly recovered. MC index, the extent of the ischemic damage calculated from mitochondrial Ca and Mg contents, was also ameliorated from 0.18 to 0.10 by the reperfusion. In this situation, the ischemic and reperfused myocardium will still be viable and be so called 'stunned myocardium'.

It is important to study what kind of the reperfusion injury will occur. Although the coronary circulation and tissue gas contents of the ischemic myocardium in 60 minute occlusion all seemed to be almost recovered by 60 minutes after reperfusion, the regional mechanical function and the mitochondrial metabolism still continued to be injured. MC index which increased to 0.37 in 60 minute occlusion was significantly deteriorated to 0.58 120 minutes after reperfusion. This index was morphologically supported by electronmicroscopic findings.

Hence, a supplementary way to protect the myocardium from ischemia is urgently needed before reperfusion when the acute myocardial infarction occurs.

With diltiazem, the regional contractility in 60 minute occlusion and 120 minute reperfusion was significantly ameliorated and was very close to the contractility in 30 minute occlusion and 120 minute reperfusion. Although the mitochondrial metabolism in 60 minute occlusion was significantly depressed after reperfusion without diltiazem treatment, it was significantly improved with diltiazem treatment. MC index in the reperfusion after 60 minute occlusion was also improved from 0.58 to 0.29 by diltiazem.

Therefore, diltiazem, a Ca$^{2+}$ antagonist, significantly ameliorated the myocardial contractility, the mitochondrial metabolism and MC index. These results were morphologically supported by electron-microscopic findings.

Therefore, it is useful to administer diltiazem to protect the myocardium from ischemia until thrombolysis or aorto-coronary bypass graft is successful.

**CLINICAL IMPLICATION**

At present, coronary reperfusion is the most effective tool for salvaging the myocardium from ischemia and preserving the left ventricular function in the early stages of acute myocardial infarction. But reperfusion can be either beneficial or deleterious. Reperfusion injury will jeopardize the salvageable myocardium. When supplementary therapy like a Ca$^{2+}$ antagonist, diltiazem, used in this study, is added as soon as possible after acute myocardial infarction, its efficacy is greatly enhanced including the cure of reperfusion injury.

Therefore, the clinical implications of coronary reperfusion, e.g. thrombolysis or aorto-coronary bypass graft, and supplementary therapy, with Ca$^{2+}$ antagonists, should be evaluated according to each treatment methods' advantages and limitations.

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