INTERMITTENT BRIEF PERIODS OF ISCHEMIA HAVE A Cumulative EFFECT AND MAY CAUSE MYOCARDIAL NECROSIS

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SUMMARY We investigated the effects of brief intermittent periods of ischemia on myocardial viability. Brief periodic coronary occlusions were produced up to 18 times by inflating and deflating the balloon of an intracoronary #2F catheter for periods of 15, 10 or 5 minutes, followed by 15-minute periods of reperfusion. Creatine kinase (CK) release, triphenyl tetrazolium chloride staining, and light and electron microscopy were used to detect the presence of myocardial necrosis. For the study of CK release, blood was taken from the great cardiac vein and the aorta before and at 5-minute intervals during each left anterior descending coronary occlusion, as well as during and 1, 5, 10 and 15 minutes after balloon deflation. In seven of 24 dogs with 15-minute occlusions, in five of 21 dogs with 10-minute occlusions, and in three of 32 dogs with 5-minute occlusions, small but distinct areas of subendocardial necrosis were present. In all dogs with morphologic proof of necrosis, there was periodic release of CK into the great cardiac vein, which peaked immediately after reperfusion, reflecting CK washout. Thus, brief periods of ischemia, which when single do not cause necrosis, have a cumulative effect and may cause myocardial necrosis. This mechanism of necrosis may be relevant clinically in patients with frequent anginal episodes. Since many dogs of this study did not have any myocardial necrosis, the findings also suggest that intermittent reperfusion has a beneficial effect and may prevent necrosis, even when total occlusion time exceeds 200 minutes.

EXPERIMENTAL STUDIES have established that to cause myocardial necrosis, an ischemic injury must be of sufficient duration and severity. Even with the severest ischemia in dogs, the injury must be present for at least 20–30 minutes before necrosis will occur. For example, if flow is restored after 15 minutes of ischemia, no myocardial necrosis will be present. However, even when ischemia is brief and myocardial necrosis does not occur, there is usually a lengthy delay in return of normal function and metabolism in the injured myocardium. Therefore, we evaluated the possibility that brief periods of ischemia before the myocardium has fully recovered from the effects of preceding episodes of ischemia may gradually increase the degree of injury to the point of nonreversibility and necrosis.

Methods

Preparation

The study was performed in mongrel dogs that weighed 20–32 kg. Anesthesia was initiated with i.v. pentobarbital, 30 mg/kg, and maintained with supplemental doses. After intubation, artificial respiration with room air was instituted with a Harvard respirator. Systemic blood pressure was measured through a catheter inserted into the femoral artery. A #5F catheter was inserted through the external jugular vein under fluoroscopic control into the coronary sinus and advanced into the great cardiac vein. The position was verified by injection of contrast material. A #7F Judkins catheter was inserted through the left carotid artery into the ostium of the left anterior descending coronary artery (LAD). A #2F Fogarty catheter was then passed through the lumen of the Judkins catheter and advanced 2–3 cm into the LAD. The Judkins catheter was withdrawn into the ascending aorta. Heparin (2000 U) was given intravenously every hour. CK activity in the blood was determined by the method of Rosalki.

Protocol

After instrumentation, the baseline ECG, pulse rate and blood pressure were recorded. In initial experiments, the intracoronary balloon was inflated for 15 minutes. The occlusion was followed by 15 minutes of reperfusion. An ECG and blood pressure were recorded during occlusion and reperfusion. The occlusion-reperfusion cycle was repeated 14–18 times, unless ventricular fibrillation supervened. Defibrillation was not attempted, for that would have altered the pattern of CK release by skeletal muscle or myocardial damage. We anticipated that the amount of CK released from the myocardium would be too small to be detected in the systemic circulation and its release limited to a very short period (seconds) after reperfusion. There-
fore, blood samples were taken directly from the great cardiac vein, which drains the myocardium supplied by the LAD. Blood samples were also obtained during and immediately after deflation of the occlusive balloon.

When the initial studies showed that necrosis might occur after repeated 15-minute occlusions, the occlusion periods were reduced to 10 minutes, and later to 5 minutes, followed by 15 minutes of reperfusion, to see whether these shorter periods also resulted in necrosis. Coronary artery patency was confirmed angiographically, and the dogs were killed with an i.v. injection of 1 g of KCl. The hearts were excised and the coronary arteries carefully dissected and examined for thrombosis or other pathologic changes. The hearts were cut into 1-cm-thick transverse slices from apex to base and stained with triphenyl tetrazolium chloride (TTC), a method shown to be sensitive and specific for myocardial necrosis. The hearts were then fixed in formalin and processed for histologic examination. In selected experiments, tissue samples were fixed in glutaraldehyde within 30 seconds of death and processed for electron microscopic examination.

Results

Figure 1 shows the results of an experiment that resulted in necrosis. Each occlusion lasted 15 minutes. During the control period and the initial cycles, the CK level does not change significantly. In cycle 13, however, there was an abrupt rise in great cardiac vein CK immediately after the balloon was deflated, which suggests a washout of CK with reperfusion. At 1 minute, the rise in CK is no longer evident. The sequence of events is repeated in cycle 14. Necrosis evidenced by CK release occurred in seven of 24 dogs subjected to repeated 15-minute occlusions, in five of 21 dogs subjected to 10-minute occlusions, and three of 32 dogs subjected to 5-minute occlusions.

In 14 of the 15 dogs that had CK release, TTC staining, histology and electron microscopy confirmed the presence of necrosis. The necrosis was limited to the subendocardium and was usually patchy (fig. 2). The exception was a dog that died of ventricular fibrillation a few minutes after CK release occurred. In this dog, the interval between necrosis and death was probably too short for loss of dehydrogenase activity, and thus, TTC evidence of necrosis, to become apparent, or for histologic and electron microscopic changes diagnostic of necrosis to evolve. The cumulative ischemic period resulting in necrosis was 20–180 minutes. The duration of ischemia necessary to cause necrosis was generally inversely proportional to the severity of ischemia, as indicated by the magnitude of the ST-segment elevation and the QRS prolongation in the ECG obtained from the site of maximal apical impulse.

Discussion

The results of this study indicate that brief ischemic episodes, which singly would not cause irreversible cell injury, can lead to myocardial necrosis when repeated. Repeated ischemic episodes of 5, 10 or 15

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**Table 1. Necrosis Resulting from Repeated, Brief Periods of Ischemia**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Duration of occlusions (min)</th>
<th>Cycles until CK release (n)</th>
<th>Total ischemic time until CK release (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>7</td>
<td>105</td>
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<td>11</td>
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<td>8</td>
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</tr>
<tr>
<td>15</td>
<td>5</td>
<td>12</td>
<td>60</td>
</tr>
</tbody>
</table>

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**Figure 1. Creatine kinase (CK) levels in the great cardiac vein (GCV) after repeated 15-minute occlusions of the left anterior descending coronary artery. The CK levels did not increase after the initial cycles. After the thirteenth occlusion, however, there was an abrupt, marked increase, which indicates myocardial necrosis due to the cumulative effects of repeated ischemic insults. The abrupt rise and rapid return to baseline levels represent a washout phenomenon.**
Although within 20 minutes produced myocardial necrosis in 15 of 77 dogs, as evidenced by CK release and morphologic changes.

Studies in dogs have shown that if reflow occurs within 20 minutes of coronary occlusion, no necrosis results. Although the ECG returns rapidly to normal, many other variables remain abnormal for prolonged periods of time. DeBoer et al. showed that after only 15 minutes of coronary occlusion, the ATP levels remain abnormally low for 72 hours. This depression of high-energy phosphate production could greatly impair the myocardium's ability to repair sublethal struc-

tural alterations and resume normal function. After a 15-minute coronary occlusion, ultrastructural abnormalities, such as glycogen depletion and cytoplasmic vacuolization, may persist for 3 days; systolic mechanical function may remain abnormal for 7 days and diastolic function for 10 days. These abnormalities occur in the absence of myocardial necrosis. It is not surprising, therefore, that new insults superimposed on a compromised myocardium have a cumulative effect, which may eventually cause irreversible injury. In 62 of the 77 dogs, however, necrosis did not develop, despite cumulative ischemic periods as long as 210 minutes. This finding suggests that the 15-minute reperfusion periods allowed a significant degree of recovery in most dogs, such that relatively long periods of myocardial ischemia could be tolerated without necrosis. It is not clear from our study why necrosis developed in some dogs and not in others. In general, dogs with necrosis had electrocardiographic signs of severe ischemia, marked ST-segment deviation or marked intraventricular conduction delay. In many dogs with only mild electrocardiographic changes, necrosis did not develop. It seems likely, however, that the rate and degree of recovery during the reperfusion periods were influenced not only by the severity of ischemia, but also by other factors, possibly metabolic.

The patterns of CK release observed in this study are also of interest. We anticipated that only small foci of myocardium might become necrotic and that systemic CK levels might not increase appreciably. Thus, to avoid dilutional effects, blood samples were taken from the great cardiac vein rather than the systemic venous system. CK elevations after reperfusion were brief and returned to baseline levels within 1 minute. The fact that 14 of 15 dogs with increases in CK levels in blood had morphologic proof of necrosis is strong evidence supporting the conventional belief that CK release indicates severe membrane damage and necrosis, and that reversibly ischemic cells do not release CK.

**Clinical Implications**

The findings of this study suggest that in man, brief episodes of ischemia may result in myocardial necro-
sis. In our experimental model, ischemia was usually severe, since it was produced by complete coronary occlusion and associated with ST-segment elevation. Unlike our model, in classic angina pectoris, the arteries may only be stenotic; the ECG shows ST-segment depression, which indicates less severe ischemia, probably limited to the subendocardium. The fact that patients with severe coronary disease and a history of angina pectoris without infarction often have small foci of myocardial fibrosis at necropsy suggests that anginal episodes alone may sometimes produce myocardial necrosis. In man, the severely stenotic arteries may not allow the same degree of recovery during reperfusion as in our canine model, in which the coronary arteries were normal.

Unstable angina or variant angina with frequent attacks associated with ST-segment elevation may be more analogous to our model. Mild elevations of CK and positive technetium-99 pyrophosphate scans have been observed in patients with unstable angina, which suggests that repeated ischemic episodes have a cumulative effect and result in necrosis. The findings of our study also indicate that the absence of abnormally elevated serum CK levels does not mean that no myocardial necrosis has occurred in patients with frequent episodes of angina pectoris. The volume of necrotic myocardium may be too small to result in detectable elevations of systemic CK levels. If myocardial necrosis can occur as a result of repeat episodes of angina pectoris, prevention and treatment may not only relieve symptoms, but may prevent or reduce myocardial damage.

The lack of necrosis observed in the majority of dogs in this study shows that intermittent reperfusion, even for very brief periods, may have a substantial beneficial effect in delaying myocardial necrosis. In patients who have intermittent pain and fluctuating ST-segment abnormalities, the onset and rate of necrosis may be delayed, and interventions designed to be instituted early, such as thrombolytic therapy and coronary artery bypass surgery, might be effective even more than a few hours after the onset of ischemia.

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