Favorable Remodeling Enhances Recovery of Regional Myocardial Function in the Weeks After Infarction in Ischemically Preconditioned Hearts

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Background—In a previous study, we found that recovery of segment shortening in the ischemic zone of conscious, chronically instrumented rabbits was significantly better in ischemically preconditioned than control animals after 72 hours of reperfusion. However, although this period of reperfusion was felt to be sufficient to allow recovery from stunning, regional function was disproportionately low for the size of the infarcts.

Methods and Results—To further characterize the recovery of left ventricular regional function, rabbits were chronically instrumented with a balloon occluder around a branch of the left coronary artery and a pair of ultrasonic crystals to monitor segment shortening in the ischemic zone. The preconditioned group had 1 cycle of 5-minute occlusion/10-minute reperfusion before a 30-minute occlusion, whereas control rabbits experienced only the 30-minute occlusion. All monitored segments were either dyskinetic or akinetic during the 30-minute occlusion. There was no difference in function between the 2 groups until 24 hours of reperfusion. At 72 hours, systolic shortening in control hearts averaged only 5% of the preischemic value, whereas shortening was 29% of baseline in preconditioned hearts. By day 21, systolic shortening averaged 26% in control hearts and 65% in preconditioned hearts ($P<0.02$) and appeared to have reached a plateau. Infarct size was 31.4±2.8% and 15.5±2.1% in control and preconditioned hearts, respectively. Moreover, in ischemically preconditioned hearts, the recovery of regional function was better than in controls for any given amount of microinfarction in the myocardial segment between crystals ($P=0.02$).

Conclusions—The progressive improvement in preconditioned hearts is most consistent with favorable remodeling in the ischemic zone, which the preconditioning process seems to accentuate. **(*Circulation*. 2000;102:579-583.)**

Key Words: myocardial infarction ■ remodeling

It is universally appreciated that ischemic preconditioning salvages ischemic myocardium.[1] However, until recently it was not known whether this reduction in infarct size actually translated into a significant improvement in myocardial function of in situ preconditioned hearts. Most studies in the rabbit,[2–5] dog,[6,7] and pig[8] have reported that ischemic preconditioning preceding coronary occlusions of 30 to 60 minutes had no salutary effect on postischemic segment shortening, wall thickening, or left ventricular systolic or end-diastolic pressures when measurements were confined to the first few hours after reperfusion. The logical explanation was that the surviving tissue had not had sufficient time for recovery from myocardial stunning in an acute protocol.[9] Accordingly, we chronically instrumented rabbit hearts with ultrasonic crystals to measure regional myocardial function during a 72-hour period after release of a coronary occlusion, which should have been sufficient time for recovery from stunning.[10] Although the degree of left ventricular systolic dysfunction was comparable in control and ischemically preconditioned groups immediately after reperfusion, recovery by 72 hours was significantly better in the latter. Because only 10% of the ischemic zone was infarcted in preconditioned hearts, it was surprising that segment shortening after 72 hours of reflow had recovered to only 44% of baseline. Thus, the recovery of function was disproportionately less than the degree of myocardial salvage. The time course of changes in segment shortening in our previous study suggested that a plateau of function had not been reached at 72 hours of reperfusion, particularly in the preconditioned group. We therefore sought to determine whether regional function would continue to improve when the observation period was prolonged to 3 weeks. We also tested whether histological abnormalities of the recovering myocardium might correlate with the degree of recovery.

On the basis of previous experience, it seemed unlikely that any residual stunning would persist beyond 72 hours. There-

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fore, it was hypothesized that any alteration in regional function beyond 72 hours would most likely be the result of active remodeling in the ischemic region. In most instances in the past, remodeling has been regarded as a detrimental event resulting in worsening of function. Virtually all previous studies have focused on the myocardium remote from a transmural infarct. In conscious dogs with nontransmural infarction, however, Kambayashi et al[11] noted that gradual hypertrophy of viable epicardial myocardium overlying infarcted subendocardium actually improved recovery of regional contractile function. The present study also looks at the function of the ischemic region. The clinical implications are obvious. After myocardial infarction, prognosis is determined by left ventricular systolic function.[12–14] Infarct limitation by ischemic preconditioning would have an impact on a patient's prognosis only if it caused a significant improvement in regional function.

Methods

This study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (Department of Health and Human Services, NIH publication 85-23, revised 1985).

Surgical Preparation

As detailed previously,[10] New Zealand White rabbits of either sex were anesthetized with pentobarbital sodium (30 mg/kg IV), orally intubated, and ventilated with 100% oxygen. The heart was exposed through a left thoracotomy, a prominent epicardial branch of the left coronary artery was surrounded by a balloon occluder, and a pair of 1-mm ultrasonic piezoelectric crystals (Sonometrics) was implanted into the mid left ventricle in the region made cyanotic by balloon inflation. The crystals were aligned to ensure that the transmission pathway between them was perpendicular to the long axis of the left ventricle. The balloon occluder and crystal wires were exteriorized near the spine. The chest was closed in layers, and ECG leads were attached to subcutaneous tissues at either end of the thoracotomy incision.

Protocol

Rabbits were allowed to recover for 1 week and then were returned to the laboratory. The crystal wires were attached to a sonomicrometer (Triton) and the ECG wires to an amplifier. As already described,[10] a phonocardiogram recorded with a hand-held microphone placed on the chest of the awake rabbit was used to register the first and second heart sounds to permit precise timing of the beginning and end of systole. All rabbits underwent a 30-minute balloon inflation and coronary occlusion. ST-segment elevation confirmed transmural myocardial ischemia. Rabbits were randomized to control and preconditioned groups. The latter also underwent a single cycle of 5-minute coronary occlusion/10-minute reperfusion before the 30-minute occlusion. ECG, phonocardiogram, and segment length tracings were recorded at baseline, during the preconditioning cycle of ischemia/reperfusion, continuously during the 30-minute coronary occlusion and first hour of reflow, and then at 1, 3, 7, 10, 14, 17, and 21 days of reperfusion. After the last recording, rabbits were reanesthetized with pentobarbital sodium.

Measurement of Infarct and Risk Zone

The heart was reexposed, excised, and hung on a Langendorff apparatus. The aorta was flushed retrogradely with saline to remove blood from the coronary arteries. The coronary artery was ligated at the occluder site, and Zn/Cd sulfide fluorescent microspheres were injected into the perfusate to demarcate the risk zone as the nonfluorescent region. The crystals were removed, and a 5-0 suture was placed at the surface opening of each tract to demarcate its location. The heart was frozen, sliced into 2-mm-thick slices from apex to base, and stained with triphenyltetrazolium chloride (TTC) to identify infarcted tissue. Infarct and risk zones of each slice were traced onto plastic overlays, and areas were planimetered. Volumes were calculated and summed. Infarction is presented as a percentage of the risk zone.

Histological Studies

After infarct size measurement, all slices were stored in 10% formalin. The slices containing the crystal sites were selected and analyzed. Tissue processing was done by an individual blinded to the group identity of the slice. Occasionally, the 2 crystal sites were on adjacent slices. In this situation, the slices were lined up, one was marked to indicate the position of the second crystal, and this latter slice was then used for the histological studies. A drawing of each selected slice was made, and after review of the previously drawn infarct and risk zone outlines, representative regions of normally perfused and noninfarcted risk-zone myocardium were selected and marked on the drawing along with an indication of the segment of myocardium between the crystals.

Samples were excised from the slices by use of the above map and embedded in paraffin. Slices 5 μm thick were stained with hematoxylin-eosin and analyzed at low power with standard light microscopy (DMLB-Microscope, Leica). Infarction was identified by loss of myocytes and increased density of collagen fibers and imaged with a video camera (Leica) mounted on the microscope. Areas of infarction were determined by use of an image analysis program (dhs Bilddatenbank V4.01, Leica) and expressed as a percentage of the total area analyzed. To confirm identification of infarcts, adjacent thin slices were stained with picrosirius red, which enhances birefringence of collagen fibers when illuminated with polarized light. These images were compared with those made from the slices stained with hematoxylin-eosin.

Segment Shortening

Segment shortening was calculated from the segment length recordings as $100\times(EDL-ESL)/EDL$, where EDL and ESL are end-diastolic and end-systolic length, respectively. The calculations were normalized for the baseline segment shortening. Thus, segment shortening of 100% indicates shortening equivalent to the preischemic value, whereas 0% signifies akinesis and a negative segment shortening implies systolic bulging.

Statistics

Values are presented as mean±SEM. One-way ANOVA with repeated measures and post hoc testing with the paired Student's *t* test were used to test for differences within groups, and unpaired Student's *t* test was applied for analysis of infarct size. Paired and unpaired *t* tests were used to analyze differences in segment end-diastolic lengths. Differences in segment shortening between groups were analyzed with 2-way ANOVA with repeated measures. Regression analysis was used for correlation of the extent of microinfarction between the 2 crystals with segment shortening. Differences of segment shortening in the groups were tested by ANCOVA using the percentage of microinfarction as a covariate. A value of $P<0.05$ was considered to be significant.

Results

Six control and 6 preconditioned rabbits completed the protocol and were euthanized 3 weeks after coronary occlusion and reperfusion. During the 30-minute coronary occlusion, 4 rabbits, 3 control and 1 preconditioned, developed ventricular fibrillation and were quickly shocked with 100 J of direct current. All were resuscitated and were included in the study. One other preconditioned rabbit developed ventricular fibrillation and suffered a spinal cord injury during defibrillation, necessitating euthanasia. In addition, 1 preconditioned rabbit died 11 days after reperfusion, and 2 control rabbits died at 15 days. These deaths were related to pro-

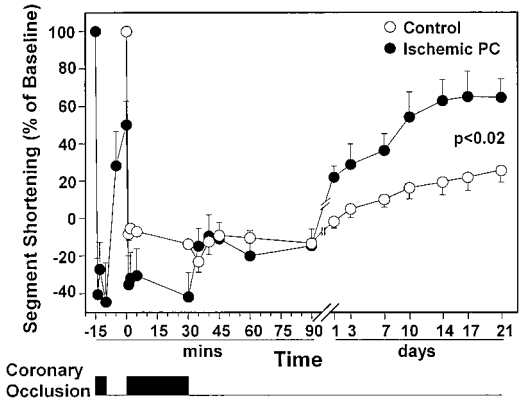

Figure 1. Summary of segment shortening data as percentage of baseline for control and preconditioned (PC) groups. In the latter, segment shortening averaged 50.3% before long occlusion. Systolic bulging was evident in both groups at end of 30-minute coronary occlusion. During first hour of reperfusion, there was little difference between groups. A difference began to emerge after 24 hours and continued to become more obvious during first 10 days of reperfusion. By 3 weeks, segment shortening appeared to have reached a plateau in the 2 groups. At that time, segment shortening averaged 64.7% of baseline in preconditioned hearts, significantly greater than 25.6% in control hearts ($P<0.02$).

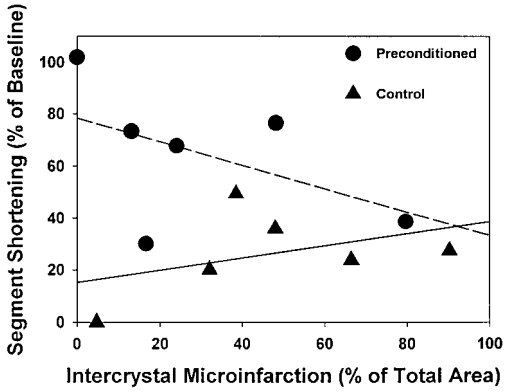

Figure 2. Segment shortening as a function of histological microinfarction of myocardial segment between crystals in preconditioned and control hearts. Regression lines are drawn for both groups and are significantly different ($P=0.02$). For preconditioned animals, there is an inverse relationship between segment shortening after 3 weeks of reperfusion and documented microinfarction ($r=0.50$), whereas relationship in control rabbits is much flatter.

tracted diarrhea and dehydration. These latter 3 rabbits did not complete the protocol and therefore were excluded from the final analysis.

There was no difference in either end-diastolic segment length (5.4±0.2 and 4.6±0.3 mm) or absolute segment shortening (14.14±1.34% and 13.10±2.89%) in control and preconditioned hearts at baseline before any intervention. Because of the similarity of these measurements and to facilitate comparison between groups, the remaining segment-shortening data have been normalized for these baseline measurements.

As shown in Figure 1, preconditioned hearts were stunned after the cycle of brief ischemia/reperfusion, and average segment shortening was 50.3±12.6% of baseline immediately before the longer occlusion. During the 30-minute coronary occlusion, there appeared to be more systolic bulging in preconditioned hearts. At the end of the occlusion, all control and 5 of the 6 preconditioned hearts bulged during systole, whereas the remaining preconditioned heart was akinetic. As previously reported,[10] there were no immediate differences between the 2 groups after release of the coronary occlusion and subsequent reperfusion. There was a difference at day 1 (Figure 1), which progressively became more apparent and significant. But by day 21, systolic function had still not returned to the preischemic value. Average segment shortening in preconditioned hearts was 64.7±9.8%, whereas it was 25.6±6.5% in control animals ($P<0.02$). After 3 weeks, segment shortening appeared to have reached a plateau in the 2 groups.

As already noted, end-diastolic lengths in the preconditioned and control hearts were not different at baseline. Whereas there was no significant change in end-diastolic length of the intercrystal segment during reperfusion in preconditioned hearts (from 4.6±0.3 mm at baseline to 5.0±0.7 mm at 3 weeks), the shrinkage in control hearts was significant (from 5.4±0.2 mm at baseline to 4.9±0.3 mm at 3 weeks, $P<0.05$).

Although fibrosis and remodeling may have influenced quantification of infarction in these hearts, infarct size was measured. Infarction averaged 31.4±2.7% of the risk zone in control hearts and 15.5±2.1% in preconditioned animals ($P<0.005$). These averages are not different from those seen previously at 72 hours, a time when significant fibrosis or resorption would not have occurred.[10]

The implanted crystals were generally in regions free of gross infarction detected with TTC staining. Remodeling over the 3-week reperfusion period in the preconditioned group resulted in a marked increase in regional wall motion, such that the difference between preconditioned and nonpreconditioned hearts was now quite striking. To further explore the relationship between infarction and function, histological studies were done to look for microinfarction. Microinfarction might not be detectable with the TTC stain, but it could profoundly affect function. No microinfarction was found outside the risk zone. In those viable regions of the risk zone that stained red with TTC, microinfarction was minimal and averaged only 8.6±3.9% and 6.2±4.0% of the total myocardial area examined in control and preconditioned groups, respectively.

Segment shortening is plotted against the extent of intercrystal microinfarction in Figure 2 for preconditioned and control hearts. The plot reveals an almost flat relationship between percent infarction and function for the control hearts. The correlation coefficient for the regression line is 0.42. The preconditioned hearts showed the expected inverse relationship between percent microinfarction and regional function, with a regression coefficient of 0.50. ANCOVA of segment shortening using microinfarction as a covariate revealed a strong group effect ($P=0.02$), implying that the preconditioned hearts were behaving differently from the nonpreconditioned hearts for any level of microinfarction. This analysis suggests that the nonpreconditioned hearts had a contractile lesion in the surviving myocardium that rendered it hypody-

namic. Apparently, ischemic preconditioning not only reduces the extent of infarction but also causes the salvaged myocardium to have improved mechanical function.

Discussion

The present data support and extend the conclusions of an earlier study.[10] In the previous study, preconditioning had improved the recovery of function 72 hours after reperfusion over that in the control hearts, but the recovery was disproportionately small compared with the degree of salvage obtained. The present study reveals that there is a steady improvement in mechanical function in the weeks that follow reperfusion, particularly in the preconditioned group. The final level of function was only mildly depressed below that expected for the extent of gross infarction in preconditioned hearts, resulting in a dramatic improvement in the latter. Function in the preconditioned hearts was inversely proportional to the amount of microinfarction between the sonomicrometer crystals. Conversely, analysis of microinfarction in the control hearts suggested that the latter suffered a contractile deficit that was unrelated to the degree of infarction between the crystals, implying that the surviving tissue no longer contracted normally.

As seen in Figure 1, segment shortening appears to reach a plateau by 3 weeks, which implies little expectation of further improvement. Most data would suggest that stunning would have been rectified within 72 hours of reperfusion. Therefore, other factors besides recovery from stunning must be influencing function. Certainly, a remodeling process is well known to occur after myocardial infarction in both experimental animals and humans.[15–17] Postinfarct remodeling has traditionally been regarded as a detrimental process, but the term itself is neutral and can describe both favorable and unfavorable changes.[18] In the present investigation, the protracted time course of improvement in the preconditioned hearts would indicate a progressive structural change in the tissue that resulted in a favorable remodeling effect in the ischemic zone. Such favorable remodeling has been observed before when hypertrophy of surviving myocardium overlying an infarct caused progressive restoration of regional function in canine hearts.[11] Because we concentrated on only the surviving tissue within the ischemic zone, we have no data on possible changes in function in the remote regions.

Only small improvement was seen in the nonpreconditioned group. It is noteworthy that there was shrinkage of the intercrystal myocardial segment in the control but not preconditioned hearts, suggesting that remodeling was qualitatively different in the 2 groups. The factors that determine whether remodeling will be favorable or unfavorable remain to be determined. Preconditioning per se could have accentuated the favorable remodeling in the present study, and our microinfarct data would support this hypothesis; or favorable remodeling could simply depend on the extent of infarction. In the study by Kambayashi et al,[11] hearts were not ischemically preconditioned, and favorable remodeling was still seen. However, all dogs were pretreated with acepromazine and buprenorphine for sedation and analgesia before coronary occlusion. The latter drug is an opioid with documented δ-receptor affinity.[19] Because such agonists are known to protect and precondition the heart,[19,20] it is possible that the dogs in Kambayashi's investigation were indeed preconditioned before coronary occlusion, thus perhaps accounting for the remarkable recovery of regional function in the ischemic zone after 3 weeks of reperfusion, as was seen in our data.

After ligation of the mid left anterior descending coronary artery and second diagonal branch in sheep with resulting anteroapical infarction, there was an immediate deterioration in circumferential and longitudinal segmental shortening in noninfarcted myocardium adjacent to the thinned, infarcted zone, with only partial recovery during the 6-month follow-up period.[21] Left ventricular end-diastolic volume increased out of proportion to the concomitant eccentric hypertrophy, and ejection fraction fell. These regional abnormalities in contractile function may reflect increased wall stress in tissue remote from the infarct[22] and may contribute to global dilatation and dysfunction. Such unfavorable remodeling has often been documented in postischemic hearts of animals and humans and may have been present in the nonischemic tissue in the present study.

Enhanced ACE activity[23] and angiotensin II levels[24] have been observed in myocardium surrounding infarcted tissue, and angiotensin II, through AT_1 receptors, can stimulate cardiac fibroblasts[25–28] and increase interstitial fibrosis in remote noninfarcted segments of the heart.[29–32] Conversely, ACE inhibitors,[29] AT_1 receptor blockers,[30,33–35] and aldosterone synthesis antagonists[35] can attenuate fibrosis in noninfarcted zones of myocardium. These agents inhibit remodeling and improve survival after myocardial infarction in experimental animals as well as humans.[36–39] After myocardial infarction in mice, inhibition of matrix metalloproteinases, which degrade extracellular matrix and are increased in infarcted tissue and border regions,[40] is associated with less remodeling and better systolic function.[41] Future studies will determine the role of ACE and matrix metalloproteinases in our model.

We looked at function only in the ischemic zone, whereas most past studies have concentrated on function in the zones remote from a transmural infarct. We did not measure function in these remote regions of the heart and therefore cannot comment on it. It is possible that unfavorable remodeling in those areas would have degraded regional function over the course of the study.

In our original study, there was a striking gap between the amount of surviving myocardium and the degree of functional recovery. Although the gap is narrower by 3 weeks, its continued presence is intriguing. The preconditioned group had 15% of the risk zone infarcted but had a 35% contractile deficit. The difference could be related to altered geometry, because the noncontracting infarct may actually impede regional function through a tethering effect.[22,42–44]

In summary, systolic function continues to improve in both control and ischemically preconditioned hearts in the first few weeks after coronary occlusion and reperfusion, and the improvement is dramatically better in the preconditioned hearts. The time course of recovery coincides with that of remodeling. Nonetheless, functional improvement still appeared to be somewhat less than expected on the basis of the extent of infarction. Finally, an analysis of microinfarction and its correlation with regional function suggested that the

surviving myocardium in ischemically preconditioned hearts was functionally better than that in nonpreconditioned hearts. The mechanistic relationship of this documented improvement to ischemic preconditioning is uncertain but deserves further exploration because of its obvious clinical importance.

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