Smaller infarct after preconditioning does not predict extent of early functional improvement of reperfused heart

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Cohen, Michael V., Xi-Ming Yang, and James M. Downey. Smaller infarct after preconditioning does not predict extent of early functional improvement of reperfused heart. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1754–H1761, 1999.—We evaluated the ability of ischemic preconditioning to restore function to salvaged myocardium in rabbits. Although ischemic preconditioning reduces infarct size, few investigators studying recovery of function after coronary occlusions lasting ≥30 min have reported any mechanical benefit in preconditioned hearts. However, because myocardial function was seldom evaluated beyond 5 h after reperfusion stunning may have masked the benefit. Accordingly, rabbits were chronically instrumented with a pneumatic occluder around a branch of the left coronary artery, a pair of 1-mm ultrasonic crystals in the myocardial territory destined to become ischemic, and electrocardiogram (ECG) leads. One week after surgery the ECG and segment length tracing were recorded at rest, during 30-min occlusion and 1 h of reflow, and again at 24, 48, and 72 h. In ischemically preconditioned rabbits, 5-min coronary occlusion and 10-min reperfusion preceded the long occlusion. The beginning and end of systole were determined by recording the first and second heart sounds with a hand-held precordial microphone. Postmortem infarct size was measured with triphenyltetrazolium chloride. During the 30-min coronary occlusion all segments became nearly akinetic or bulged during systole. After 60 min of reflow there was little return of function in either group. Between 24 and 72 h there was minimal recovery in the control group (segment shortening equals 13.3 ± 4.1% of baseline), whereas function was much better in preconditioned hearts (44.2 ± 7.4% of baseline, P < 0.02). Infarct size as a percentage of risk zone was much smaller in preconditioned hearts (10.2 ± 1.4 vs. 29.7 ± 1.8%, P < 0.001). Thus there is a gradual recovery of systolic function of reperfused myocardium after a coronary occlusion. Although early mechanical recovery is significantly better after preconditioning, it is much less than would be predicted by the reduction of infarct size.

myocardial infarction; regional myocardial function; segment shortening; stunning

IT IS WELL ESTABLISHED that myocardial infarction compromises the global function of the heart and that the resulting deficit will be proportional to the size of the infarct. Thus occlusion of a coronary artery at a proximal site will make a larger infarct and have a more deleterious effect on global myocardial performance than a more distal occlusion. It has been assumed that salvage by an anti-infarct intervention such as ischemic preconditioning would have the opposite, i.e., a beneficial, effect, and that limitation of infarct size should be accompanied by a proportional salutary effect on overall cardiac function. However, ischemic preconditioning acts to limit the transmural extent of necrosis within an anatomic risk zone rather than modify the lateral borders. The extent to which subepicardial salvage by ischemic preconditioning will contribute to systolic shortening of the involved segment has been inadequately explored. Intuitively, the contribution to overall pump function by any intervention should be directly related to restoration of segmental shortening within the ischemic zone.

Preconditioning has been demonstrated to be a potent and reliable attenuator of infarction. In most experimental models ischemic preconditioning results in up to 75% salvage of myocardium otherwise destined to become necrotic (3). Preconditioning is quite effective at minimizing the transmural extent of infarction. In the isolated rat heart the beneficial effect of preconditioning after a relatively mild ischemic insult has generally been demonstrated by documenting greater left ventricular function than in the nonpreconditioned heart (1). It has never been resolved whether this effect is the result of attenuation of stunning and/or infarction. Improved postischemic function has been much more difficult to demonstrate in in situ models of regional ischemia, probably because extensive infarction and marked stunning of any surviving myocardium render the entire segment akinetic during reperfusion. Indeed, most studies have reported that ischemic preconditioning preceding coronary occlusions of 30–60 min in the rabbit (13–15, 20), dog (18, 22), or pig (21) 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. Such a short reperfusion period would not have allowed stunned myocardium sufficient time to recover. The only exception is the study by Qiu et al. (19) in the pig, in which a benefit from preconditioning was seen immediately after reperfusion. Although the rabbit model of ischemic preconditioning has been studied extensively, the effect on long-term recovery of function in this species has yet to be investigated.

To address this issue, rabbits were chronically instrumented with sonomicrometer crystals implanted in the ischemic zone of the left ventricle. Hearts underwent 30 min of regional ischemia, and, to allow stunned segments to recover, segmental shortening was monitored during a 72-h period of reperfusion. By 72 h segmental function was indeed improved by preconditioning, but to our surprise the degree of myocardial salvage did not
predict the extent of recovery of shortening during this early period.

METHODS

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals [Department of Health and Human Services Publication No. (NIH) 85–23, revised 1985].

Surgical preparation. New Zealand White rabbits of either sex, weighing 1.8–2.3 kg, were anesthetized with intravenous pentobarbital sodium (30 mg/kg). After endotracheal intubation rabbits were ventilated with 100% oxygen via a positive-pressure respirator (MD Industries, Mobile, AL). Body temperature was maintained near 38°C with a heating pad. All animals received an intravenous infusion of 100 mg Kefzol. Under aseptic conditions the heart was exposed through a left thoracotomy in the fourth intercostal space. After the pericardium was incised a prominent branch of the left coronary artery was identified on the surface of the left ventricle. As previously described (7) the open end of a balloon occluder fashioned from 18-gauge Tygon tubing was wedged onto a curved taper needle, which was passed through the myocardium beneath the identified artery. The balloon occluder was positioned around the artery and anchored to the myocardium. Proper functioning of the balloon was assured by observing cyanosis of the distal myocardium when the balloon was inflated with air and hyperemia when the air was aspirated and the balloon was deflated. A pair of 1-mm ultrasonic piezoelectric crystals attached to sheathed 38-gauge wires (Sonometrics, London, ON, Canada) was inserted into the outer one-half of the ventricular wall in the region becoming cyanotic with balloon inflation. Because of variable anatomy of surface vessels, the crystals were placed from ~3 to 8 mm apart. The balloon occluder and crystal wires were exteriorized near the spine. Two electrocardiogram (ECG) leads were attached to subcutaneous tissues at either end of the thoracotomy incision and also exteriorized near the spine. In the immediate postoperative period rabbits were treated with subcutaneous buprenorphine for analgesia and intramuscular benzathine penicillin (600,000 U).

Protocol. Rabbits were allowed to recover for 1 wk and then returned to the laboratory. The crystal wires were attached to a sonomicrometer (Triton, San Diego, CA) and the ECG wires were subjected to a 5-min coronary occlusion and subsequent reperfusion in the preconditioned rabbits, and continuously during the 30-min coronary occlusion and first hour of reflow. The ECG, PCG, and segment length were then recorded at 24, 48, and 72 h of reflow. After the last recording the coronary artery was again occluded for 5 min to be certain that the ultrasonic crystals were working properly.

Measurement of infarct and risk zone. Animals were again anesthetized with pentobarbital sodium, and the left thoracotomy incision was reopened to expose the heart. The heart was excised and mounted on a Langendorff apparatus by the aortic root. The aorta and coronary arteries were perfused with saline to remove blood, and then the enucleated coronary artery was reoccluded. To demarcate the tissue at risk, fluorescent microspheres were injected into the aortic perfusate. Thus the risk zone could be identified as that region lacking fluorescence under ultraviolet light. The heart was then frozen and sliced from apex to base into 2-mm-thick slices. Slices were incubated in triphenyltetrazolium chloride to form the dye. Viable tissue stains red because of formation of formazan dye in the cell.

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Boundaries of risk zones and infarcted tissues were traced on plastic overlays, and areas were quantitated by planimetry. Volumes were calculated by multiplying areas by slice thickness. Infarct size is expressed as a percentage of the risk zone.

Segment shortening. Segment shortening was calculated from the segment length recordings as 100(EDL - ESL)/EDL, where EDL and ESL are end-diastolic and end-systolic length, respectively. The calculations were normalized for the preischemic 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 means ± SE. One-way ANOVA with repeated measures and Tukey-Kramer post hoc tests were used to test for differences within groups, and unpaired Student's t-test was applied for analysis of infarct size and end-diastolic length data (Systat, Evanston, IL). Finally, differences between groups were analyzed with two-way ANOVA with repeated measures. P < 0.05 was considered to be significant.

RESULTS

Six control and seven preconditioned rabbits completed the protocol. Three instrumented rabbits died during the first 24 h after surgery. Two additional rabbits, one in the control group and one preconditioned animal, died prematurely of noncardiac causes between the 24- and 48-h studies and were excluded. One additional rabbit never regained contractile function after preconditioning with 5 min of coronary occlusion and was also excluded. Contrary to our prior experience (5), one control and five preconditioned rabbits fibrillated during the prolonged coronary occlusion. All were successfully defibrillated with external direct current shocks of 100 J. Rabbits have a strong neuromuscular discharge with the onset of ventricular fibrillation. Thus one animal sustained a broken back when its heart fibrillated and had to be euthanized, and in a second group the wires broke at the exit site on the dorsum of the animal thus terminating the experiment.

To simplify the surgery and experimental protocol, indwelling arterial catheters were not inserted in these groups of rabbits. However, in a previous study (5) there was no difference in blood pressure between preconditioned and control rabbits either before, during, or after the 30-min coronary occlusion. In the present two groups of experimental animals there were no differences in heart rate at any time during the 72-h protocol.

Figure 2 presents PCG, ECG, and segment length tracings for one representative control animal. Coronary occlusion produced marked S-T segment elevation and systolic bulging, which persisted for the duration of balloon inflation. After reperfusion there was little change for the first hour. Over the next 3 days there was return of minimal systolic contraction, and by 72 h segment shortening in the previously ischemic region was 8.2% of the control value.

Tracings from a representative preconditioned rabbit are seen in Fig. 3. The brief preconditioning ischemia led to systolic bulging with partial return of function on reperfusion (segment shortening = 33.2% of control value). During the 30-min coronary occlusion there was again systolic bulging with little change during the first...
However, during the next 3 days there was progressive and striking return of function so that by 72 h systolic shortening had increased to 36% of baseline.

A summary of segment shortening data as a percentage of baseline for the two groups is presented in Fig. 4. In the preconditioned group return of function after the preconditioning ischemia averaged $64.4 \pm 5.6\%$ of the baseline value. Segment shortening in the preconditioned rabbits after the 5-min preconditioning ischemia was not different from that in the control group after 5 min of the 30-min coronary occlusion ($-8.3 \pm 4.5$ and $-6.6 \pm 3.4\%$, respectively), attesting to the comparability of the two groups. By the end of the 30-min coronary occlusion systolic bulging was apparent in both groups. For the first hour after reperfusion there was little difference between control and preconditioned rabbits, but with increasing reperfusion time a significant difference between the two groups appeared. By 72 h only $13.3 \pm 4.1\%$ of shortening had returned in control rabbits, whereas an average of $44.2 \pm 7.4\%$ of function was evident in the preconditioned group. This difference during late reperfusion was significant ($P = 0.02$).

Comparison between groups is facilitated by normalizing for baseline values. Without this normalization baseline segment shortening was modestly better in control rabbits ($15.2 \pm 0.7$ vs. $11.6 \pm 1.0\%, P < 0.05$). After 72 h of reperfusion absolute shortening in control rabbits averaged $2.1 \pm 0.6\%$, whereas it was $5.4 \pm 1.1\%$ in preconditioned hearts. The difference in the change of segment shortening during reperfusion between the two groups was again significant ($P < 0.05$).

To verify that the recovery of function was the result of active contraction of tissue within the ischemic zone, the coronary artery was again occluded for 5 min after the 72-h reperfusion data had been recorded. Systolic contraction declined rapidly, and the segment became akinetic or dyskinetic in all animals. Average segment
Fig. 4. Summary of segment shortening data as percentage of baseline for control and preconditioned (PC) groups. In latter segment shortening averaged 64.4% before long occlusion. Although systolic bulging was evident in both groups at end of 30-min occlusion, preconditioned rabbits demonstrated residual contraction (3.6%) after initial 5 min, whereas segment shortening averaged −8.3% at end of 5-min preconditioning ischemia. There was no difference between groups during the 1st hour of reperfusion. However, during the next 3 days there was an increasing difference between the groups, and this difference favoring the preconditioned group was significant (P = 0.02). A brief 5-min coronary occlusion at end of experiment again produced systolic bulging, attesting to reliable function of balloon occluder and implanted myocardial crystals.

shortening during coronary occlusion was $-3.7 \pm 3.0\%$ and $-4.8 \pm 2.3\%$ of baseline in preconditioned and control groups, respectively.

We tested to see whether the reperfused segment dilated over the 72-h test period. End-diastolic length increased modestly in both groups from the pres ischemic level to that measured after 72 h of reperfusion (from 4.9 ± 0.3 to 5.2 ± 0.3 and 5.7 ± 0.7 to 6.0 ± 0.6 mm in control and preconditioned rabbits, respectively), although the dilation was significant in only the preconditioned group (P = 0.044). End-diastolic length was not different in the two groups either before occlusion or after 72 h of reperfusion.

There was no difference in heart weight or risk zone volume in the groups. Ultrasonic crystals were always well within the boundaries of the risk zone. Furthermore, the crystals that were in the outer half of the left ventricular wall tended to be in spared myocardium, although in some hearts they were close to infarcted tissue. Infarction was significantly less in the preconditioned than control hearts (10.2 ± 1.4 vs. 29.7 ± 1.8% of risk zone, P < 0.001). When infarct size was plotted against segment shortening 72 h after reperfusion for the control and preconditioned groups (Fig. 5), it was apparent that the data points for the two groups did not fall on the same regression line. In fact, the regression lines for the two groups were significantly different (P < 0.01).

**DISCUSSION**

Ischemic preconditioning salvages ischemic myocardium and minimizes the size of infarcts. Although this assertion is now universally accepted, the seemingly logical corollary that the salvaged myocardium should improve regional and ultimately global cardiac function is less well established. In most models in which significant infarction occurs ischemic preconditioning does not appear to improve segmental shortening of the reperfused myocardium or hemodynamics. In the dog (18, 22), pig (21), and rabbit (13–15, 20) it has been observed repeatedly that both nonpreconditioned and preconditioned myocardium perform equally poorly after a coronary occlusion lasting >30 min despite much smaller infarcts in the latter. Differences in mechanical function between the groups in the present study became apparent only after several days of reperfusion, presumably because salvaged, but stunned, segments needed time to recover. Although preconditioned hearts were obviously better off than control hearts (Fig. 6), it is striking that segment shortening recovered to only 44% of the pres ischemic value. This was disappointing since we had anticipated an improvement in function, which would have been more proportional to the degree of salvage.

A recent study by Qiu et al. (19) revealed a very different picture from the present results. Conscious pigs were preconditioned with 10 cycles of 2-min coronary occlusion and 2-min reperfusion before a 40-min coronary occlusion. Preconditioning resulted in an immediate improvement in wall thickening over the nonpreconditioned hearts, and by 3 h of reflow thickening had recovered to 60% of baseline with little change during the next 3 days. Such an early improvement in mechanical function can be achieved in preconditioned rabbit hearts as well, but to accomplish this a much milder ischemic insult must be employed (4). In the latter study the coronary artery was occluded for only...
small infarct. One possible explanation is that the mechanisms of production of a large deficit in function by a dysfunction. At present, we can only speculate about mechanisms that may contribute to this discordance between salvage and restoration of function.

More closely mimics humans. It is obvious not known which model is effective in restoring regional function in the rabbit than in the pig. It is obviously not normal. Using a similar rabbit model, we recently reported that a large percentage of the myocytes in the surviving myocardium within the risk zone were apoptotic (8). Scattered cell death in what grossly stains as surviving myocardium could compromise overall function. Further studies will be needed to document whether this deficit is temporary and to what extent the wall motion will eventually recover in both groups.

In the present study a difference in segment shortening due to preconditioning emerged at 24 h and continued to increase for the duration of the observation period. Because no plateau in recovery was seen, it is possible that improvement would have continued. The studies were terminated at 72 h because of a concern that longer periods would result in contraction of the infarcted region that would affect our ability to measure infarct size. Thus this study cannot provide information about the ultimate degree of functional recovery in either control or preconditioned hearts. However, we were surprised how poorly the size of the infarct was able to predict the degree of residual mechanical dysfunction in preconditioned hearts at this early stage of recovery. Longer observations will be required to be more certain about the relationship between infarction and the magnitude of recovery.

One explanation for a delay in recovery of viable myocardium is possible stunning. However, most studies suggest that recovery from stunning should have been nearly complete after 72 h of reperfusion. In dogs undergoing a 5-min coronary occlusion segmental shortening returned to normal within 6 h, whereas complete recovery was delayed to 24 h if the duration of the occlusion was prolonged to 15 min (10). Bolli et al. (2) have noted that stunning the heart with six cycles of 4-min coronary occlusion and 4-min reperfusion in conscious rabbits results in an ~70% deficit in wall thickening, which is progressively and completely eliminated over the ensuing 5 h. Finally, in the baboon recovery of wall thickening after a 1-h coronary occlusion required 4 days to reach a plateau with no further improvement over the next 3½ wk (9). Because of the severity of the ischemic insult in our rabbits, it is possible that recovery from stunning in these hearts still may not have been complete. Continued improvement beyond 72 h, however, most likely would occur from remodeling of the surviving layers in an attempt to restore normal wall motion. It may be impossible to determine when stunning stops and remodeling begins.

Figure 5 reveals the relationship between infarct size and segmental shortening for the two groups. In either group there is a linear relationship between infarct size and ultimate recovery of function after 72 h of reperfusion. Not surprisingly, there was an inverse relationship between infarct size and recovery in both groups.

A more important difference between the study by Qiu et al. (19) and the present report is the degree to which wall motion was restored. Qiu et al. saw thickening fraction improve to about 70% of the preischemic value. Thus infarction of 9.4% of the risk zone caused a chronic 30% deficit in function. In our rabbits return of performance was not as encouraging. Infarct size averaged 10.2% of the risk zone (almost identical to that in the study by Qiu et al.). Yet segment shortening recovered to only 44% of the preischemic value. Hence mid-wall salvage by preconditioning was much less effective in restoring regional function in the rabbit than in the pig. It is obviously not known which model more closely mimics humans.

The present study is limited to a description of the discordance between salvage and restoration of function. At present, we can only speculate about mechanisms of production of a large deficit in function by a small infarct. One possible explanation is that the subendocardial fibers bear a disproportionate share of the systolic load and that loss of these fibers has a profound effect on overall shortening. Such an effect could be overcome by hypertrophy of the surviving fibers and shrinkage of the scar through remodeling. Another more intriguing possibility is that the salvaged tissue is not normal. Using a similar rabbit model we recently reported that a large percentage of the myocytes in the surviving myocardium within the risk zone were apoptotic (8). Scattered cell death in what grossly stains as surviving myocardium could compromise overall function. Further studies will be needed to document whether this deficit is temporary and to what extent the wall motion will eventually recover in both groups.
but the two regression lines were statistically different. The figure would suggest that for any given infarct size function would be poorer if the heart had been preconditioned. That could indicate that the myocardium salvaged by preconditioning is not functionally normal, which would explain why function was so disproportionate to infarct size in those hearts. Alternatively, a single sigmoid relationship between infarct size and regional function that passes through the data points for both groups may exist. Because there is no overlap of infarct sizes in the two groups, the two possibilities cannot be distinguished at this time. As noted previously, the direct comparison of infarct size and early recovery of function is perhaps unfair, but it is safe to suggest that this degree of early recovery in preconditioned hearts was not nearly as robust as predicted and that these observations have obvious clinical implications. Cardiologists and surgeons should not expect rapid recovery of cardiac function after emergent revascularization for acute coronary occlusion even in hearts preconditioned by preceding angina or other intervention.

It is interesting that preconditioning appeared to preserve residual shortening during the first 5 min of the 30-min ischemic period in four of the seven hearts. This effect, however, was only transient because after 30 min of ischemia all segments were either nearly akinetic or dyskinetic, and there was no difference between preconditioned and control groups. No early benefit during ischemia was seen in preconditioned hearts in patients undergoing minimally invasive coronary artery bypass graft surgery when the regional left ventricular contraction pattern was evaluated with transesophageal echocardiography (16). Wall motion abnormalities were not different during the initial 5-min preconditioning coronary occlusion and the subsequent coronary occlusion for attachment of the arterial graft. It is not clear what caused the delay in deterioration of function in our rabbits, but perhaps there is precedent. Although brief coronary occlusion results in S-T segment elevation signaling myocardial ischemia, the magnitude of S-T elevation is attenuated during subsequent coronary occlusions (6), suggesting that at least some abnormality of myocardial function manifested during ischemia can be successfully modified.

In conclusion, it is reassuring that ischemic preconditioning appears to result in more robust postischemic segmental function, albeit smaller than expected. This augurs well for the eventual clinical application of preconditioning.

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REFERENCES


