Short-term Synchronized Retroperfusion Before Reperfusion Reduces Infarct Size After Prolonged Ischemia in Dogs

Yasushi Wakida, MD; Rolf Nordlander, MD; Shiro Kobayashi, MD; Sheila Kar, MD; Roberto Haendchen, MD; Eliot Corday, MD

Background. Previous studies have demonstrated that synchronized coronary venous retroperfusion (SRP) can restore blood flow to the ischemic myocardium, resulting in infarct size reduction and improvement of the left ventricular function. Despite the nutritive blood flow achieved by SRP being relatively limited, SRP has been shown to improve washout of by-products from the ischemic myocardium. The aim of this study was to investigate whether short-term SRP immediately prior to reperfusion would attenuate the deteriorative phenomena following reperfusion.

Methods and Results. Closed-chest anesthetized dogs underwent 3 hours of left anterior descending coronary artery (LAD) occlusion. The dogs were then randomized into two groups: (1) control group (n=9), in which the occlusion was immediately followed by 3-hour reperfusion; or (2) SRP group (n=9), in which SRP was started 3 hours after occlusion and maintained for 30 minutes with sustained occlusion followed by 2.5-hour reperfusion with simultaneous discontinuation of SRP. There were no statistical differences between the groups in global hemodynamics and degree of ischemia measured by radiolabeled microspheres. Myocardial infarct size (triphenyltetrazolium method) expressed as percentage of risk area was significantly smaller in the SRP group (24±7%, mean±SEM) than in the control group (54±9%). The extent of myocardial hemorrhage expressed as percentage of infarct size was also significantly reduced in the SRP group (3±2%) compared with the control group (24±6%). The increase in end-diastolic wall thickness in the ischemic area after reperfusion assessed by two-dimensional echocardiography was significantly less in the SRP group. Blood flow measurements after reperfusion demonstrated the occurrence of no-reflow phenomenon only in the control group. Histological examination revealed extensive myocardial hemorrhages only in the control group, which extended into the nonnecrotic myocardium in four of nine hearts and extensive contraction band necrosis compared with the SRP group.

Conclusions. Short-term SRP prior to reperfusion can reduce infarct size, myocardial hemorrhage, wall swelling, and no-reflow phenomenon. The mechanism of this beneficial effect is not clear but might be due to gradual reperfusion and washout of by-products from the ischemic myocardium before fully oxygenated arterial blood reperfusion. (Circulation. 1993;88[part 1]:2370-2380.)

Key Words • reperfusion • hemorrhage • edema

Rapid restoration of blood flow to ischemic myocardium is the mainstay of current therapy and appears to be the most rational way of limiting the extent of ischemic injury following acute coronary occlusion. However, sudden antegrade reperfusion of arterial blood may cause additional myocardial injury, known as "reperfusion injury." Multiple theories have been put forward to explain this phenomenon. These include the generation of toxic reactive oxygen metabolites caused by sudden reperfusion of arterial blood with high oxygen concentration and the incomplete return of flow to ischemic areas, so-called no-reflow phenomenon. The latter have been related to granulocyte trapping, microthrombosis, and cellular and interstitial edema, all of which have been clearly associated with reperfusion.

Synchronized diastolic coronary venous retroperfusion (SRP) is a method of delivering arterial blood to ischemic myocardium through the coronary venous system. Several reports have shown reduction of ischemia and feasibility as well as safety of this technique, both experimentally and clinically. However, in most of these studies, SRP has been applied in the early minutes following coronary occlusion. There are few reports demonstrating the effectiveness and safety of retroperfusion applied in the later stages of ischemia, when more severe myocardial and endothelial cell degeneration has already taken place. It has been documented that SRP enhances washout of metabolic by-products from the ischemic zone and may actually expand the venocapillary system, thereby potentially reducing granulocyte trapping and thus the source of oxygen free radicals in the ischemic myocardium. Moreover, experimental studies using radioactive microspheres and 133Xe have demonstrated that the restoration of perfu-
ision achieved by SRP is only 10% to 60% of normal levels.\textsuperscript{7,9} This implies that reoxygenation of ischemic myocardium occurs partially and perhaps more gradually than with antegrade reperfusion.

The purpose of the present investigation was to assess the effectiveness and safety of short-term SRP in a moderately late ischemic stage (3 hours of coronary occlusion) followed by antegrade reperfusion. Our hypothesis was that washout of metabolic by-products from the ischemic zone and partial restoration of blood flow by SRP followed by conventional antegrade reperfusion would minimize known complications associated with full, sudden reperfusion, namely, reperfusion injury. Three hours of coronary occlusion in the closed-chest dog model appeared to be appropriate to test this hypothesis since it is known to cause partial necrosis of the area at risk.

**Methods**

The study was approved by the Cedars-Sinai Institutional Animal Care and Use Committee and conforms to the guidelines of the American Heart Association.

**Animal Preparation**

Twenty-two mongrel dogs weighing 19 to 28 kg (average, 22.8 kg) were premedicated with morphine sulfate (2 mg/kg) IM. Anesthesia was induced by IV administration of sodium thiopental (25 mg/kg), and after endotracheal intubation, it was maintained with a mixture of air and oxygen with ethrane (3% to 5%) using an anesthesia machine (Ventura 75000, Dupaco Inc, Arcadia, Calif) and a Harvard respirator. Respiration volume and rate were adjusted to keep arterial blood gases within physiological range (pH 7.35 to 7.45; P\textsubscript{O\textsubscript{2}}, 100 to 200 mm Hg; P\textsubscript{CO\textsubscript{2}}, 35 to 45 mm Hg). Electrodes were attached to the left chest wall and limbs to provide continuous ECG monitoring.

An 8F catheter was inserted in the inferior vena cava from the right femoral vein for injection of fluid and drugs. Heparin was given as a bolus injection (8000 IU) before instrumentation and subsequently supplemented with 1500 IU every hour. A 7F catheter was inserted into the descending aorta from the left femoral artery to measure systemic blood pressure and to withdraw reference blood samples for myocardial blood flow calcula-
tions. Under fluoroscopic guidance, an 8F microtip transducer catheter (Millar Instruments, Inc, Houston, Tex) was introduced via the right carotid artery and positioned in the left ventricle for continuous monitoring of left ventricular pressure. A 7.5F retroperfusion catheter (see below) was inserted into the coronary sinus via the left jugular vein and advanced into the great cardiac vein in the vicinity of the anterior interventricular vein. To obtain arterial blood for SRP, an 8F catheter with an end-hole and multiple sideholes was inserted into the descending aorta via the left femoral artery. For injection of radioactive microspheres, a 4F Corday-Lang occlusion catheter (American Edwards Laboratories, Irvine, Calif) was inserted transseptally into the left atrium through a 7F Teflon sheath (44 cm, USCi, Billerica, Mass) using the Brockenbrough technique.\textsuperscript{11} A Mullins 7F pediatric transseptal catheter introducer set (USCi) was used for this purpose, and the tipped balloon was inflated with 0.5 mL Renografin. An 8F modified left coronary guiding catheter was inserted via the left carotid artery to intubate the left main coronary artery.

**Experimental Protocol**

After baseline measurements of global hemodynamics, two-dimensional echocardiography and regional myocardial blood flow (RMBF) (see below), a 3F Fogarty arterial embolectomy balloon catheter (American Edwards Laboratories, Anasco, Puerto Rico) was inserted into the left anterior descending coronary artery (LAD) through the guiding catheter and positioned just proximal to the midventricular diagonal branch. The balloon was then inflated to 1.5 atm using an angioplasty in-deflator device (USCi) to maintain the LAD occluded for 3 hours. Angiography was performed to confirm complete coronary occlusion, and images were recorded onto videotape for documentation of its exact location. At 2.5 hours after LAD occlusion, additional measurements including RMBF were performed. At this point, the dogs were randomly assigned to either sudden reperfusion (control group) or to 30 minutes of SRP followed by sudden reperfusion (SRP group). Dogs in which adequate SRP, ie, a flow rate of more than 30 mL/min with a coronary sinus venous pressure of less than 60 mm Hg,\textsuperscript{5} could not be performed, and dogs with a transmural blood flow of more than 40 mL·min\textsuperscript{-1}·100 g\textsuperscript{-1} in the central ischemic zone during LAD occlusion were to be excluded from the final analyses.

In the control group, the occlusive intracoronary balloon was abruptly deflated 3 hours after LAD occlusion, and the catheter withdrawn from the coronary artery. Patency of the LAD was confirmed by angiography. Sequential (15, 30, 60, 120, and 180 minutes after reperfusion) hemodynamic and echocardiographic recordings were performed during the reperfusion period. Microsphere injections were done at 5, 30, and 180 minutes after reperfusion. In the SRP group, SRP was started 3 hours after LAD occlusion and maintained during 30 minutes with sustained occlusion of the LAD, followed by antegrade reperfusion in a manner identical to the control group, with simultaneous discontinuation of SRP. In this group, sequential hemodynamic and echocardiographic recordings were obtained at 15 and 30 minutes of SRP and at 15, 30, 60, 120, and 150 minutes following the start of antegrade reperfusion. Microsphere injections were performed at 5, 30, and 150 minutes after reperfusion (Fig 1).

After the final measurements were obtained (6 hours from the start of the experiment), the LAD balloon catheter was reinserted, and the LAD was reoccluded at exactly the same site, using the video recording as a guide. Monastral blue dye was then injected into the left atrium for determination of the risk area, and the animals were killed using an overdose of potassium chloride under deep anesthesia.

No antiarrhythmic drugs were used throughout the protocol to avoid a possible effect on infarct size.\textsuperscript{12}

**Synchronized Retroperfusion System**

SRP was achieved by using an ECG synchronized catheter-pump system (Retroperfusion System, Inc, Costa Mesa, Calif), previously described in detail.\textsuperscript{3-7} Briefly, arterial blood is shunted from the right femoral artery into the great cardiac vein using a triple-lumen...
Fig 1. Plots of individual values of myocardial blood flow in the endocardial region of the ischemic center. In the control group (left), there was a significant reduction in the blood flow during the reperfusion period (REP.). PRE.OCC indicates preoclusion; and 180°OCCL, 180 minutes of coronary occlusion.

7.5F SRP catheter with a balloon located 1 cm from its distal end. The balloon is inflated in diastole by ECG triggering, and arterial blood is propelled into the myocardium subserved by the occluded LAD. During systole, balloon deflation facilitates drainage of coronary venous blood. One lumen of the catheter is used for monitoring of coronary venous pressure. SRP was initiated at a flow rate of 10 mL/min, which was rapidly increased until peak coronary venous pressure reached 40 to 60 mm Hg, giving flow rates between 40 and 200 mL/min.

Global Hemodynamics

Heart rate, mean aortic blood pressure, end-diastolic and peak systolic left ventricular pressures, and its first derivatives (+dp/dt and -dp/dt) as well as coronary sinus pressure were recorded on an electrophysiologic recorder (Honeywell Inc, Pleasantville, NJ).

Two-Dimensional Echocardiography

To obtain adequate short-axis echocardiographic images of the left ventricle, all dogs were placed in the right lateral position using a modified table with a center cut for appropriate transducer positioning and imaging, as previously described. The echo transducer (3.5 MHz) was placed underneath the right chest wall, and a mid-papillary muscle-level short-axis cross-section image of the left ventricle was recorded with a built-in video recorder (Ultramark 8, Advanced Technology Laboratories Co, Bothell, Wash). A computer-assisted system was used to measure average (3 beats) end-diastolic (EDWT) and end-systolic wall thickness (ESWT). Systolic wall thickening (%WTH) was calculated by the following equation: %WTH=100×(ESWT−EDWT)/EDWT. Percent change in end-diastolic wall thickness (%ΔEDWT) was calculated as follows: %ΔEDWT=100×(observed EDWT−baseline EDWT)/baseline EDWT. All measurements were performed blindly by one investigator without knowledge of SRP treatment.

Regional Myocardial Blood Flow

Measurements of RMBF were obtained using approximately 2 million radioactive microspheres (15-μm diameter, EJ DuPont de Nemours & Co, Inc, Boston, Mass) diluted in 7 mL of 0.9% saline solution, which was injected into the left atrium, followed by 10 mL saline flush. For each injection, the isotope was chosen randomly from 46Sc, 57Co, 99mTc, 103Ru, 51Cr, and 113Sn-labeled microspheres. As RMBF is calculated by the reference withdrawal method, reference blood samples were obtained from the descending aorta at a constant rate (3.88 mL/min) using a Harvard withdrawal pump beginning 1 minute before the microsphere injection and maintained for a period of 5 minutes.

After the dogs were killed, the hearts were cut from apex-to-base into 1-cm-thick transverse sections parallel to the atroventricular groove. The third myocardial slice with the papillary muscles included was sectioned into three zones: a central risk zone (middle of risk area) and lateral and a septal risk zones (immediately adjacent to the central risk zone but within the risk area). The nonrisk posterior wall (opposite wall) was equally sectioned into three zones. Each section was further subdivided into epicardial, midmyocardial, and endocardial regions, and reference blood samples were then counted with appropriately selected energy windows using a Modular Automatic Gamma Counting System (TM Analytic, model 1185, Elk Grove Village, Ill) and corrected for background and crossover by a computer software program (Microsphere Measurement System, Micrad Inc, Knoxville, Tenn). RMBF was calculated by the formula: RMBF=RBF×TCOUNTS/(BCOUNTS×WEIGHT)×100, where RBF is the reference blood sample withdrawal rate (3.88 mL/min), TCOUNTS is tissue counts, BCOUNTS is blood counts, and WEIGHT is sample weight (g). Blood flows are expressed in mL·min⁻¹·100 g⁻¹.

After calculating RMBF in each of the three myocardial layers, transmural blood flow in the center of the risk or nonrisk areas as well as in the total risk or nonrisk areas (center plus border zones) was also calculated.

Pathology

The hearts were sliced from apex to base into 1-cm-thick transverse sections parallel to the atroventricular groove. The areas of the left ventricle at risk represented by areas unstained by blue dye were traced on a transparent sheet in all myocardial slices. Macroscopically hemorrhagic areas were also delineated on the same sheet. The myocardial slabs were then incubated in triphenyl-tetrazolium chloride (TTC) solution, and the infarcted areas represented by nonstained TTC areas were traced on a separate transparent sheet. These areas were quantitated blindly using a computer-aided planimetry system. Risk area expressed as percentage of the left ventricle, infarcted area as percentage of the risk area, and hemorrhagic area as percentage of the infarcted area were calculated. All slices except the one taken for microsphere blood flow measurements were fixed in 10% formalin. After more than 3 days of fixation, these sections were used for histologic processing by conventional methods, and the risk area of the basal surface site of the second slice was stained with hematoxylin and eosin. The histopathologic assessments were done blindly by a pathologist unaware of SRP treatment.
Table 1. Global Hemodynamic Variables

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CONT indicates control group (n=9); SRP, synchronized retroperfusion group (n=9); Occl, after coronary occlusion; REP, reperfusion; SRP, during synchronized retroperfusion; HR, heart rate; MAP, mean aortic pressure; LVPSP, left ventricular peak systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt, maximal rate of rise of left ventricular pressure; −dP/dt, maximal rate of decline of left ventricular pressure; and CSP, coronary sinus pressure.

Values are given as mean±SEM. *P<.01 vs control.

Statistical Analyses

All data are expressed as the arithmetic mean±SEM. Comparisons of data between the two groups were performed by Student's t test. Repeated-measures ANOVA was used for analysis of serial results (hemodynamics, echocardiographic findings, and RMBF). If there was a statistically significant difference with repeated-measures ANOVA, further analysis was performed by Tukey-Kramer multiple-comparison test. To estimate the relationship between transmural blood flow in the risk area and infarct size, least-squares linear regression and correlation coefficients were calculated followed by ANOVA for testing the significance of the regression. The comparison of regression lines between the two groups were performed by analysis of covariance (ANCOVA). Statistical significance was considered at P<.05.

Results

All 22 dogs survived the entire study protocol. No dog developed ventricular fibrillation. One dog assigned to the SRP group was excluded from analyses. This dog, weighing 20 kg, had a narrow and small coronary sinus and developed unacceptably high coronary sinus pressure (>70 mm Hg) at SRP flow rates of only 10 mL/min. Of the remaining 21 dogs, 3 dogs (1 from the control group and 2 from the SRP group) had rich collateral blood flow indicated by the microsphere blood flow measurements (transmural blood flow to the center of the ischemic zone was 66, 46, and 63 mL·min⁻¹·100 g⁻¹, respectively), and there was no necrosis after TTC staining. These 3 animals were also excluded from further analysis. Thus, 9 dogs in the control group and 9 dogs in the SRP group were included in the final analysis.

Global Hemodynamics

The global hemodynamic results are summarized in Table 1. There were no statistically significant differences in any of the hemodynamic parameters between the two groups at preocclusion baseline, 180 minutes of occlusion, or during the SRP and/or reperfusion period except for coronary sinus pressure (CSP) during SRP, where the peak CSP increased from 13.8±2.1 to 44.8±3.3 mm Hg with a mean flow rate of 118±19 mL/min. In both groups, there was an increase in heart rate and decrease in left ventricular peak systolic pressure, +dp/dt, and −dp/dt.
after coronary occlusion and during reperfusion, but there were no significant differences between the groups. Although +dp/dt and −dp/dt tended to be lower in the SRP group after coronary occlusion, this difference did not reach statistical significance.

**Regional Myocardial Blood Flow**

RMBF measurements are tabulated in Table 2. There were no statistically significant differences between the groups at any time during the experiment. At 180 minutes of coronary occlusion, blood flow in the risk area was significantly higher in the epicardial layer compared with the endocardial layer in both groups as expected. However, collateral blood flow distribution in the different layers as well as transmural blood flow in the central and in the total risk area were similar among the groups. Five minutes after antegrade reperfusion, the endocardial blood flow returned to preocclusion values in both groups. However, in the control group, the endocardial blood flow decreased further 30 and 180 minutes after reperfusion (P<.05) as opposed to no significant reduction in the SRP group, even though...
Echocardiographic Findings

Table 3 summarizes the end-diastolic wall thickness and the percent systolic wall thickening measured by two-dimensional echocardiography. At 180 minutes of occlusion, there was a significant reduction in the end-diastolic wall thickness and the percent systolic wall thickening in the risk area compared with the baseline preocclusion values. The percent systolic wall thickening showed negative values at this time in both groups, indicating systolic wall thinning (−10.6 ± 4.7 and −19.9 ± 6.6 in the control and SRP groups, respectively). After 15 minutes of reperfusion, the end-diastolic wall thickness increased in the control group relative to the 180-minute occlusion value (+108%) and did not change significantly during the following 180 minutes of reperfusion. In contrast, in the SRP group, the end-diastolic wall thickness increased by only 39% 15 minutes after the initiation of SRP and by 74% 15 minutes after antegrade reperfusion. The increases in end-diastolic wall thickness in the SRP group were, however, significantly less than the corresponding increases in the control group at any time during reperfusion (Fig 3).

**Table 3. Two-Dimensional Echocardiographic Findings**

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<th>Pre.Occl</th>
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**CONT** indicates control group (n=9); **SRP**, synchronized retroperfusion group (n=9); **EDWT** end-diastolic wall thickness; **ESWT**, end-systolic wall thickness; **I**, ischemic region; **n**, nonischemic region; **%WThl**=(ESWT−EDWT)/EDWT×100; **Pre.Occl**, preocclusion; **Occl**, coronary occlusion; **REP**, retroperfusion; **Rep.**, reperfusion; and **SRP**, synchronized retroperfusion.

Values are given as mean±SEM. *P<.05, †P<.01 vs control, §P<.05 vs control 15'REP.
Microscopic Findings

Microscopic examination of the ischemic (risk) regions revealed extensive myocardial hemorrhages in the control hearts, which extended into the nonnecrotic myocardial tissue in 4 of the 9 cases (Fig 6). The hearts from the control animals showed extensive areas of contraction band necrosis, particularly in the peripheral infarcted area, whereas the hearts from the SRP-treated animals showed only patchy areas of contraction band necrosis, with predominant areas of coagulation necrosis.

Discussion

In this study, using an anesthetized dog model, reperfusion after 3 hours of coronary occlusion resulted in approximately 50% necrosis of the area at risk, with 25% of this area comprised by moderately severe hemorrhagic infarcts. Short-term (30-minute) SRP prior to reperfusion reduced infarct size by approximately 50%, with a reduction in the hemorrhagic area of 85% compared with the control group. As there were no hemodynamic differences between the groups, except increased coronary venous pressure during SRP treatment, at any time during the experiment, a direct beneficial effect of synchronized retroperfusion on ischemic-reperfused myocardium is suggested. This also indicates that sudden reperfusion may cause additional myocardial injury, which, in this experimental model, could be reduced by treatment with retroperfusion prior to full antegrade reperfusion.

Whether reperfusion per se causes necrosis of nonlethally injured cells is still a matter of controversy. Formation of oxygen free radicals, accumulation and activation of granulocytes in the ischemic tissue with subsequent plugging of the microcirculation, and calcium overload have all been implicated as possible mechanisms for reperfusion injury. Oxygen free radicals appear to play a major role and have been studied in a variety of conditions. It has been well established that oxygen free radicals are highly cytotoxic in vitro16,17 or in small animals in vivo18 and that they are rapidly formed in ischemic-reperfused myocardium experimentally19-22 and clinically.23,24 However, whether oxygen free radicals are similarly deleterious to the ischemic myocardium in large animal species is still a matter of
debate. In several studies, the presence of this phenomenon has been well documented by using pharmacologic agents known to scavenge free radicals from ischemic-reperfused myocardium. However, one of the criticisms against such studies is that the agents themselves might affect infarct size, particularly when TTC or similar staining techniques are used for infarct size estimation. In the present study, only the mode of reperfusion was modified without addition of any chemical compound, and substantial infarct size reduction estimated by TTC staining could be demonstrated. Effects of similar magnitude on infarct size have been shown after coronary venous retroinfusion of pharmacologic agents with oxygen free radical scavenging properties. If TTC staining is reliable, such findings strongly indicate the occurrence of reperfusion-induced cell necrosis in large animals (see "Study Limitations").

Possible Mechanisms for Prevention of Reperfusion Injury by Retroperfusion

The mechanism behind the beneficial effect of SRP observed in this study is not clear. However, several possibilities should be discussed. The mode of flow restoration to the acutely ischemic myocardium afforded by SRP is unique and may be more suitable for the critical early phases of tissue reoxygenation.

Washout of toxic products. One possible beneficial effect obtained by SRP pretreatment is enhanced washout of potentially toxic products from the ischemic myocardium, such as oxygen free radicals and white blood cells. Although none of these products were measured in the present study, a washout effect of retroperfusion has previously been described. Other studies have shown infarct size reduction after reperfusion using pressure-controlled intermittent coronary sinus occlusion, a technique that is known to enhance the washout of metabolites from ischemic myocardium with very little blood perfusion. Similar results have also been achieved by antegrade reperfusion using acellular fluids.

Staged reperfusion. In this study, the retrograde blood flow achieved by the SRP pump was 118 ± 19 mL/min (mean ± SEM), which is almost equivalent to the normal antegrade blood flow for 100 g of myocardium. However, some studies have indicated that retroperfusion restores only 20% to 60% of the preclosure blood flow to the capillary bed of ischemic myocardium. Chang and colleagues used digital subtraction angiography to estimate the amount of retrograde blood flow obtained by this system. They found that retroperfusion resulted in 20% to 30% of antegrade flow. A similar study, using a washout method, has shown that only 10% of the antegrade flow was achieved by retroperfusion.
findings indicate that retroperfusion is associated with partial and perhaps more gradual tissue reoxygenation. Previous studies have shown that sudden, full reperfusion of ischemic myocardium may cause additional myocardial damage and that it is particularly associated with severe myocardial edema, hemorrhage, and possibly additional cell necrosis. Controlled, staged antegrade reperfusion, on the other hand, has been shown to decrease these phenomena in experimental animals. A more recent study showed that infarct size reduction after controlled reperfusion was associated with attenuation of no-reflow phenomenon, similar to those found in the present study.

**Oxygen concentration in reperfused blood.** There are a number of studies associating tissue injury to the amount of oxygen free radical formation in reperfused myocardium, which appears to be highly related to the oxygen content of the reperfusate. The actual oxygen content of retroperfused blood reaching the ischemic myocardium is probably lower than that of antegrade reperfused arterial blood, because the former is a mixture of the retroperfused arterial blood and venous blood drained from nonischemic areas. This may possibly result in less oxygen free radical formation and consequently less tissue damage.

**Changes in Regional Myocardial Blood Flow, Edema, and Myocardial Hemorrhage**

Another possible mechanism associated with beneficial effects of retroperfusion compared with antegrade reperfusion is expansion of the venocapillary system, which may improve the microcirculation by preventing neutrophil plugging of the capillaries. The present results showing that myocardial blood flow after reperfusion of the ischemic endocardium decreased over time in the control group as opposed to no change in the SRP group support this presumption. The midmyocardial blood flow, however, showed a lack of hyperemic response in the control group. Although this finding was not statistically different compared with the SRP group, it may indicate an important mechanism for the subsequent infarct development as this part of the myocardium corresponds to distribution of contraction band necrosis seen, dominantly, in the control group.

Reduction of tissue edema may also contribute to the beneficial effects of retroperfusion. Myocardial edema occurs within minutes after conventional antegrade reperfusion and has been shown to correlate with no-reflow phenomenon as well as with the ultimate transmural extent of the infarct. Partial restoration of blood flow by retroperfusion prior to full antegrade reperfusion is the most likely explanation for the smaller change in end-diastolic wall thickness observed in the SRP group, suggesting less myocardial edema. Myocardial hemorrhage associated with reperfusion could also contribute to myocardial edema and possibly also to additional cell damage. Although previous studies have indicated that reperfusion-induced hemorrhage is exclusively limited to the infarcted tissue, a recent clinical study has indicated that myocardial hemorrhage following reperfusion with urokinase can extend beyond the infarcted tissue. This has been clearly documented in four of the nine control dogs in this study without any thrombolytic agents. Myocardial hemorrhage was significantly reduced by pretreatment with retroperfusion.

**Clinical Implications**

In this experimental study, reperfusion was intentionally delayed and initiated only 3 hours after coronary occlusion. This resulted in fairly large infarctions in the control group, whereas the extent of necrosis in the intervention group was substantially reduced. This model of delayed intervention is probably more realistic in clinical practice because reperfusion therapies can seldom be implemented in the first hours after severe ischemia has actually taken place. Since the safety of retroperfusion has been well established clinically, investigation of this intervention prior to conventional antegrade reperfusion strategies such as coronary angioplasty or emergent coronary bypass surgery may be justified.

Moreover, this technique can also be used for delivery of pharmacologic agents into the ischemic myocardium. Experimental studies have indicated that timing and mode of drug administration, such as oxygen free radical scavengers, can be a critical factor in pharmacologic reduction of ischemic-reperfusion injury. Further studies, however, are needed to assess the applicability of these technologies in humans.

**Study Limitations**

One limitation of the present study is the short observation period after reperfusion, which was limited to 3 hours. It has been claimed that reperfusion injury is only a process in which necrosis is accelerated and that the final infarct size is not affected by reperfusion. However, this concept is based on results from studies on the effects of oxygen free radical scavengers, which have shown discrepant results in terms of infarct size reduction between short- and long-term reperfusion protocols. More recent studies, however, have also shown controversial results using protocols with extended reperfusion periods. A critical factor in this controversy appears to be related to timing and mode of drug administration rather than the varying reperfusion periods.

Another limitation is the difference in time course between the two groups. The control group underwent 3 hours of coronary occlusion and 3 hours of reperfusion, whereas the SRP group underwent 3.5 hours of coronary occlusion (with SRP given during the last 30 minutes of this period) followed by 2.5 hours of antegrade reperfusion. Although the difference in occlusion time would tend to favor the control group, the shorter period of observation following antegrade reperfusion in the SRP group could have a bearing on the process of reperfusion injury. If one, however, considers that SRP is in essence a form of partial reperfusion, the "reperfusion" period is equal between the groups. A longer reperfusion protocol would certainly help to clarify this point.

Measurements of RMBF by microsphere technique during retroperfusion may have some limitations as already trapped microspheres could be washed out by the retroperfusion itself, thus underestimation of the actual blood flow. This limitation should be kept in mind when the difference between the intercepts of linear regression (Fig 5) is analyzed. However, the tendency toward a lesser difference in infarct size with increased flow and greater difference in infarct size with
less flow should not be affected by this limitation, speaking in favor of a true beneficial effect of SRP.

Estimation of infarct size by the TTC staining method has been criticized in protocols using relatively short periods of reperfusion. In a recently published study, however, a good correlation was found between estimated infarct size measured by TTC and histopathology using a similar experimental protocol.

None of the discussed mechanisms regarding the effects of SRP prior to antegrade reperfusion for limitation of reperfusion injury have been specifically studied in this experimental model. Therefore, we cannot know which of these mechanisms actually played the major role in preventing the reperfusion injury by the SRP pretreatment. If one considers this technique just a method for partial restoration of myocardial perfusion, the question arises whether antegrade partial reperfusion will result in a similar reduction of infarct size or whether reperfusion per se has some additional benefit. To address this question, a comparison between SRP pretreatment and partial antegrade reperfusion prior to full antegrade reperfusion in the similar ischemic model as the present study should be performed.

In conclusion, this experimental study demonstrates that short-term (30 minutes) coronary venous retroperfusion of autologous arterial blood after 3 hours of coronary occlusion and preceding full antegrade reperfusion significantly reduced myocardial edema, myocardial hemorrhage, and no-reflow phenomenon. This resulted in a reduction of the infarct size after 2.5 hours of reperfusion. These findings also suggest that reperfusion may cause tissue injury and that it can be prevented by modifying the mode of blood flow restoration to acutely ischemic myocardium.

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