

Cardioprotective Role of Remote Ischemic Perconditioning in Primary Percutaneous Coronary Intervention

Enhancement by Opioid Action

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Objectives We sought to determine the potential of remote ischemic perconditioning (RIPC), and its combination with morphine, to reduce reperfusion injury in primary percutaneous coronary interventions.

Background Remote ischemic post-conditioning is implemented by applying cycles of ischemia and reperfusion on a remote organ, which result in release of circulating factors inducing the effects of post-conditioning on the myocardium.

Methods A total of 96 patients (59 men) were enrolled. The patients were randomized to groups as follows: 33 to each treatment group (Group A: RIPC; Group B: RIPC and morphine) and 30 to the control group (Group C). Measures of efficacy were achievement of full ST-segment resolution (primary), and reduction of ST-segment deviation score and peak troponin I during hospitalization.

Results A higher proportion of patients in Groups A (73%) and B (82%) achieved full ST-segment resolution after percutaneous coronary intervention, compared with control patients (53%) ($p = 0.045$). Peak troponin I was lowest in Group B, 103.3 ± 13.3 ng/ml, in comparison to peak levels in Group A, 166.0 ± 28.0 ng/ml, and the control group, 255.5 ± 35.5 ng/ml ($p = 0.0006$). ST-segment deviation resolution was $87.3 \pm 2.7\%$ in Group B, compared with $69.9 \pm 5.1\%$ in Group A and $53.2 \pm 6.4\%$ in the control group ($p = 0.00002$). In paired comparisons between groups, Group B did better than the control group in terms of both ST-segment reduction ($p = 0.0001$) and peak troponin I ($p = 0.004$), whereas Group A differences from the control group did not achieve statistical significance ($p = 0.054$ and $p = 0.062$, respectively).

Conclusions These findings demonstrate a cardioprotective effect of RIPC and morphine during primary percutaneous coronary intervention for the prevention of reperfusion injury. This is in agreement with observations that the beneficial effect of RIPC is inhibited by the opioid receptor blocker naloxone. (J Am Coll Cardiol Intv 2010;3:49–55) © 2010 by the American College of Cardiology Foundation

The strategy of urgent reperfusion of ischemic myocardium in patients with acute ST-segment elevation myocardial infarction (STEMI) has led to a significant improvement of outcomes, because early reperfusion minimizes the extent of damage of heart muscle and, ideally, preserves the function of the heart. However, reperfusion after a prolonged period of ischemia damages the myocardium, through a process known as ischemia-reperfusion injury (1). Post-conditioning is defined as repetitive cycles of briefly interrupted reperfusion applied at the onset of establishing reflow and has been shown to significantly improve outcome following an episode of

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ischemia and to reduce the size of myocardial infarct, when applied at the time of reperfusion (2,3). Ischemic post-conditioning has also been associated with long-term improvement of left ventricular function after STEMI (4). Remote ischemic post-conditioning may be achieved by

Abbreviations and Acronyms

mPTP = mitochondrial permeability transition pores

PCI = percutaneous coronary intervention

RIPC = remote ischemic perconditioning

STEMI = ST-segment elevation myocardial infarction

Tnl = troponin I

applying cycles of ischemia and reperfusion not in the heart, but rather in a remote organ, which results in release of circulating factors inducing the effects of post-conditioning on the myocardium (5).

A growing body of evidence suggests that mitochondrial permeability transition pores (mPTP) may play a major part in post-conditioning, on the basis of such observations as mPTP opening during early reperfusion and

modification of reperfusion phenomena by genetic alterations of mPTP (6). Inhibition of mPTP opening has been reported to be an important mechanism underlying the protective effect of post-conditioning (7), and the signaling pathway initiated by opioid receptor activation has been shown to lead to this cardioprotective effect, by targeting mitochondria (8). In a similar manner, Piot et al. (9) showed that the immunosuppressant drug cyclosporine, a potent mPTP-opening inhibitor, administered shortly before primary reperfusion in patients with STEMI, was associated with smaller infarct size.

The aim of the present study was to assess the potentially additive effect of remote ischemic perconditioning (RIPC) and opioid receptor activation, by morphine administration, both applied at the onset of reperfusion, on specific myocardial damage indicators. (We use “perconditioning” instead of “post-conditioning” to indicate that ischemic conditioning was applied while ischemia persisted—a few minutes before balloon inflation—and during the time of

reperfusion.) The clinical context of the study was primary percutaneous coronary intervention (PCI) in patients with acute STEMI.

Methods

Population and treatment assignment. Ninety-six patients were enrolled and were submitted to primary PCI. Inclusion criteria were: 1) acute STEMI (ST-segment elevation >1 mm, in more than 2 contiguous leads, with accompanying chest pain); 2) symptom onset not more than 6 h before presentation; and 3) age 35 to 75 years. Patients in Killip class VI or with moderate/severe renal failure (serum creatinine higher than 1.5 mg/dl) were excluded. Patients provided informed consent. The study protocol was approved by the Institutional Review Board.

The patients were randomly assigned to 3 groups: Group A was submitted to RIPC, Group B received both RIPC and intravenous morphine infusion at the onset of reperfusion, and Group C was control patients (no RIPC was applied and no morphine was used at the onset of reperfusion). All patients received standard pharmacological treatment as per the institutional guidelines for STEMI.

Coronary angiography and PCI. All patients underwent coronary angiography by the Judkins technique, and PCI was performed at the presumed culprit lesion. The choice of guidewires, balloons, stent types (bare-metal or drug-eluting), and stent characteristics was made by each individual operator according to common laboratory practice. Lesions were crossed with a guidewire and pre-dilated, with inflation of a balloon to its nominal pressure, corresponding to the diameter of the vessel. Additional inflations were

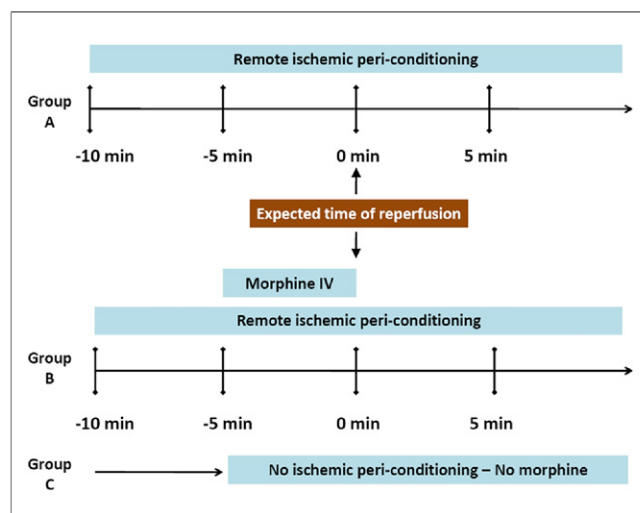


Figure 1. Study Plan

Plan of remote ischemic perconditioning application and administration of morphine in patients submitted to primary coronary intervention, according to the randomization group. The 0 point in the time axis represents the estimated time of first balloon inflation. IV = intravenous.

employed if necessary. Stents were then deployed according to established techniques.

RIPC and morphine infusion. We induced RIPC by inflating a blood pressure cuff placed on the upper limb to 20 mm Hg above systolic arterial pressure for 4 min and deflating the cuff for 4 min; 3 such cycles were performed, beginning 10 min before the estimated time of first balloon inflation. (It is evident that the exact time of first balloon inflation could not be pinpointed beforehand, nor would it be ethical to delay balloon inflation in order to keep an exact period of time from the beginning of RIPC; as a consequence, RIPC began *approximately* 10 min before the first balloon inflation). Patients belonging in Group C (control) also had a manometer cuff placed on their upper arm, which was inflated up to a pressure level of 20 mm Hg *below* their diastolic blood pressure.

Morphine was administered as an intravenous slow infusion of 5 mg of morphine sulfate, beginning 5 min before the estimated time of first balloon inflation. Control patients received a similar infusion of normal saline solution, without addition of morphine. **Figure 1** illustrates a diagram of the treatment plan per randomization group.

Efficacy measures. The measures used to assess the efficacy of the employed methods of reducing reperfusion injury (RIPC and morphine infusion) were the achievement of full ST-segment resolution (primary end point), and the percent reduction of ST-segment deviation score and peak cardiac troponin I (TnI) levels attained during hospitalization. The number of patients achieving full ST-segment resolution was the pre-specified primary efficacy measure.

Resolution of ST-segment deviation after reperfusion has been shown to be associated with better outcome after STEMI (10,11). In the present study, ST-segment deviation score was measured in 12-lead electrocardiograms, calculated as the sum (in millimeters) of ST-segment deviation (elevation or depression) at 80 ms after the J-point in all 12 leads. ST-segment deviation score was measured at presentation and 30 min after PCI, by 2 physicians blinded as to the patients' data, and the mean value of the 2 assessments was used. ST-segment resolution was calculated as a percentage: ratio of the reduction in ST-segment deviation score from presentation to half an hour after PCI over the ST-segment deviation score at presentation ×

Table 1. Baseline Epidemiological and Clinical Features of the Study Population

| | Group A (n = 33) | Group B (n = 33) | Group C (n = 30) | p Value |
|---|---------------------|---------------------|---------------------|---------|
| Demographic and clinical parameters | | | | |
| Age, yrs | 62.9 ± 11.1 | 63.9 ± 11.2 | 61.2 ± 10.9 | NS |
| Men | 20 (61%) | 21 (64%) | 18 (60%) | NS |
| Smoking | 24 (73%) | 26 (79%) | 20 (67%) | NS |
| Dyslipidemia | 16 (48%) | 14 (42%) | 12 (40%) | NS |
| Diabetes | 10 (30%) | 11 (33%) | 9 (30%) | NS |
| Hypertension | 16 (48%) | 15 (45%) | 13 (43%) | NS |
| Serum creatinine, mg/dl | 1.1 ± 0.2 | 1.2 ± 0.2 | 0.9 ± 0.3 | NS |
| Previous MI | 5 (15%) | 4 (12%) | 5 (17%) | NS |
| History of previous PCI | 4 (12%) | 4 (12%) | 3 (10%) | NS |
| CAD history | 10 (30%) | 8 (24%) | 7 (23%) | NS |
| Ejection fraction | 0.45 ± 0.07 | 0.48 ± 0.06 | 0.44 ± 0.05 | NS |
| Treatment* | | | | |
| Beta-blocker | 32 (99%) | 30 (98%) | 30 (100%) | NS |
| CCB | 5 (15%) | 6 (18%) | 4 (13%) | NS |
| ACEI | 28 (85%) | 27 (82%) | 28 (93%) | NS |
| ARB | 1 (3%) | 2 (6%) | 1 (3%) | NS |
| Statin | 33 (100%) | 33 (100%) | 30 (100%) | NS |
| ST-segment deviation score, mm | 18.6 ± 9.4 | 19.0 ± 9.2 | 20.0 ± 11.4 | NS |
| PCI-related parameters | | | | |
| Time from onset of pain to first balloon inflation, min | 193 ± 23 | 198 ± 28 | 192 ± 35 | NS |
| Time from presentation to first balloon inflation, min | 46 ± 12 | 48 ± 14 | 50 ± 15 | NS |
| TIMI flow grade 3 post-PCI | 31 (94%) | 30 (91%) | 28 (93%) | NS |
| Values are mean ± SD or n (%). Group A, remote ischemic perconditioning only; Group B, remote ischemic perconditioning and morphine infusion; Group C, no remote ischemic perconditioning or morphine infusion (control patients). **Treatment* refers to the patients' in-hospital MI management. ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; CAD = coronary artery disease; CCB = calcium channel blocker; MI = myocardial infarction; NS = not significant; PCI = percutaneous coronary intervention; TIMI = Thrombolysis In Myocardial Infarction. | | | | |

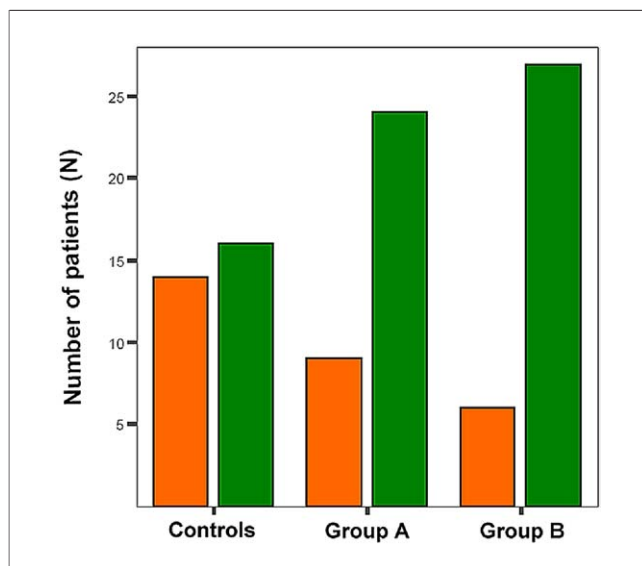


Figure 2. Primary End Point Achievement per Group

Number of patients showing full resolution ($\geq 80\%$) of ST-segment deviation after percutaneous coronary intervention in each randomization group. The difference was significant for both Groups A and B, when compared to the control group ($p = 0.045$ in the Kruskal-Wallis test; $p = 0.015$ in the Jonckheere-Terpstra test). Slightly more patients in Group B than in Group A achieved full resolution, but the difference between these 2 groups was insignificant. Resolution of ST-segment changes: **orange bars** = incomplete resolution; **green bars** = full resolution.

100% (i.e., [(ST-segment deviation at presentation – ST-segment deviation post-PCI)/ST-segment deviation at presentation] \times 100%). Full ST-segment resolution was defined as 80% or more reduction of ST-segment deviation score.

Serum levels of TnI were measured using a commercially available quantitative assay (Abbott Laboratories, Abbott Park,

Illinois), in blood samples collected using standard venipuncture techniques on presentation and at regular intervals during hospitalization. Blood was collected into pyrogen-free blood collection tubes and centrifuged, within 15 min from collection, using a centrifuge with an integrated refrigeration system (at 4°C , $3,000\text{ g}$ for 20 min). All serum samples thus obtained were analyzed in no more than 1 h.

Statistical analysis. Continuous variables are summarized as mean value \pm SEM, unless otherwise indicated, and categorical variables as counts and/or percentages. Comparisons of continuous variables were made using analysis of variance, applying a general linear model, with post-hoc Bonferroni correction for multiple comparisons. In order to assess the distributional assumptions for analysis of variance—normality and equal variance assumptions—the Kolmogorov-Smirnov and the Levene tests were used, respectively. For variables whose distribution differed significantly from the normal and/or equal variance could not be assumed, nonparametric analysis was performed (Kruskal-Wallis and Jonckheere-Terpstra tests). Categorical variables were cross-tabulated and the Fisher exact test was used for comparisons. Statistical significance for all statistical tests was defined as $p < 0.05$. All statistical analyses were performed with the SPSS version 15.0 statistical software package (SPSS, Inc., Chicago, Illinois).

Results

Study population and treatment. Ninety-six patients (59 men, age 62.7 ± 11.1 years) were enrolled, fulfilling the criteria outlined in the Methods section. Thirty-three patients were randomized to each treatment group (Group A: RIPC; Group B: RIPC and morphine infusion). A third group of control patients (Group C: no RIPC or morphine infusion) included 30 patients. All 3 groups were equivalent

Table 2. Peak TnI Levels and Percent Resolution of ST-Segment Changes in the 3 Randomization Groups

| | n | Mean | SD | SEM | 95% Confidence Interval for Mean | | Minimum | Maximum | F Statistic | p Value |
|---------------------------------------|----|-------|-------|------|----------------------------------|-------------|---------|---------|-------------|---------|
| | | | | | Lower Bound | Upper Bound | | | | |
| Peak TnI, ng/ml | | | | | | | | | | |
| Control group | 30 | 255.5 | 194.5 | 35.5 | 182.9 | 328.1 | 20 | 906 | 8.06 | 0.0006 |
| Group A | 33 | 166.0 | 160.8 | 28.0 | 109.0 | 223.0 | 25 | 801 | | |
| Group B | 33 | 103.3 | 76.5 | 13.3 | 76.2 | 130.4 | 24 | 421 | | |
| Total | 96 | 172.4 | 161.5 | 16.5 | 139.7 | 205.1 | 20 | 906 | | |
| Resolution of ST-segment deviation, % | | | | | | | | | | |
| Control group | 30 | 53.2 | 35.2 | 6.4 | 40.0 | 66.3 | 0 | 90 | 12.06 | 0.00002 |
| Group A | 33 | 69.9 | 29.1 | 5.1 | 59.6 | 80.2 | 0 | 100 | | |
| Group B | 33 | 87.3 | 15.4 | 2.7 | 81.8 | 92.7 | 20 | 100 | | |
| Total | 96 | 70.6 | 30.6 | 3.1 | 64.4 | 76.8 | 0 | 100 | | |

Group A, remote ischemic perconditioning only; Group B, remote ischemic perconditioning and morphine infusion; Group C, no remote ischemic post-conditioning or morphine infusion (controls). F statistic and p values refer to the differences between the 3 groups.

SD = standard deviation; SEM = standard error of the mean; TnI = troponin I.

as far as basic demographics and clinical parameters were concerned (Table 1). The time from symptom onset until the first balloon inflation was 193 ± 23 min in Group A, 198 ± 28 min in Group B, and 192 ± 35 min in Group C ($p = \text{NS}$). There were no significant differences in the time from presentation at the emergency room until first balloon inflation between the 3 groups (Table 1). Inflation of the brachial manometer cuff was used to start RIPC, which was started on average 8 ± 2 min before first balloon inflation. ST-segment deviation score before PCI was correlated to peak TnI levels (Pearson correlation index: 0.606; $p < 0.001$).

Efficacy measures. When the resolution of ST-segment-changes was studied as a binary variable (with full resolution being considered as the primary end point of the study), there was a significantly higher proportion of patients in Groups A (73%) and B (82%) who achieved full resolution half an hour after PCI than in the control group (53%) ($p = 0.045$). Figure 2 illustrates the ascending number of patients with full resolution of the ST-segment changes, from Group C (control) to Group A, to Group B.

As far as the resolution of ST-segment changes was concerned, as measured by the percent reduction of ST-segment deviation score half an hour after PCI, and compared with the pre-PCI ST-segment deviation score, there was a marked difference in favor of patients in Group B (Table 2). More specifically, ST-segment deviation score was reduced by $87.3 \pm 2.7\%$ in Group B, compared with $69.9 \pm 5.1\%$ in Group A and $53.2 \pm 6.4\%$ in the control group (overall $p = 0.00002$) (Fig. 3A). With the patients divided into tertiles according to the percent reduction of ST-segment deviation (ST-segment resolution tertiles), a significant difference was observed in the distribution of ST-segment resolution tertiles in the 3 treatment groups (Fig. 4): most patients (61%) of Group B belonged to the higher tertile of ST-segment resolution compared with 10% of control patients ($p = 0.00018$), whereas in Group A, 30%, 48.5%, and 21.5% of patients belonged to the lower, middle, and higher tertiles of ST-segment resolution.

The data for peak TnI levels and degree of ST-segment return to baseline (expressed as percent reduction in ST-segment deviation score) are summarized, per randomization group, in Table 2.

The overall peak TnI level was 172.4 ± 16.5 ng/ml. Peak TnI was lowest in patients who received both RIPC and morphine (i.e., Group B), 103.3 ± 13.3 ng/ml, in comparison to those who received only RIPC (i.e., Group A), 166.0 ± 28.0 ng/ml, and the control group (i.e., Group C), 255.5 ± 35.5 ng/ml (overall $p = 0.0006$) (Fig. 3B). If the 2 active treatment groups (Group A plus Group B) were aggregated and compared with the control group, peak TnI levels would be 134.7 ± 15.9 ng/ml versus 255.5 ± 35.5 ng/ml ($p = 0.003$).

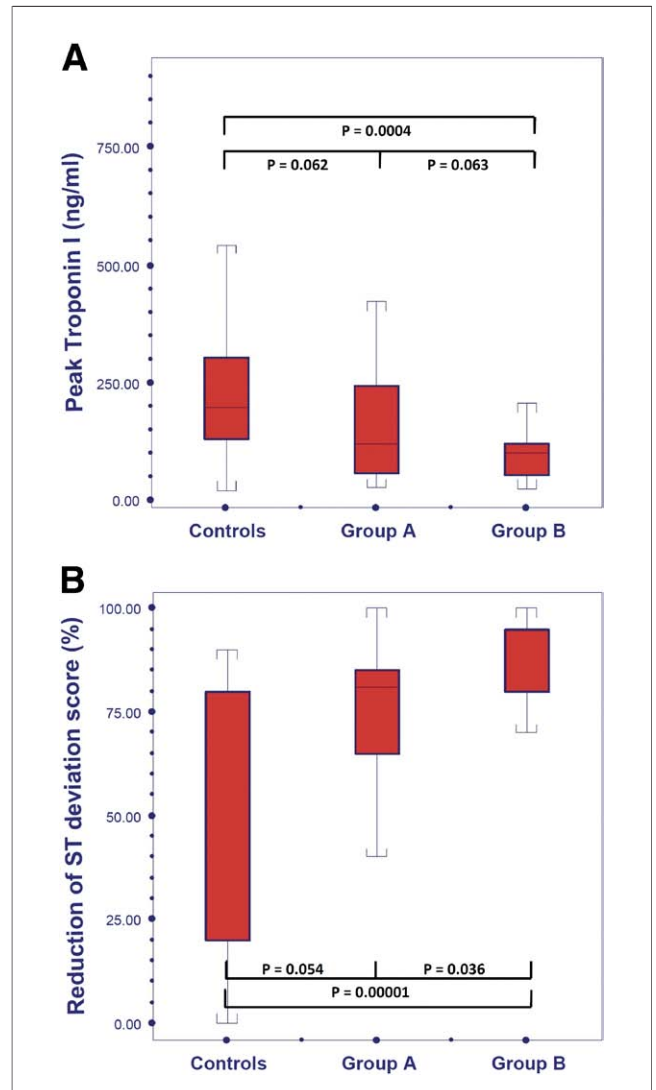


Figure 3. Box Plot Graphs for ST-Segment Resolution Score and Peak TnI

Box plot graphs of peak troponin I (TnI) levels (A) and ST-segment resolution (B) in the 3 study groups. In both cases, remote ischemic perconditioning plus morphine fared significantly better than no treatment. (The whiskers indicate range, excluding outliers.)

Discussion

The principal finding of the present study was the higher proportion of STEMI patients achieving full resolution of ST-segment deviation 30 min after primary PCI, when RIPC was applied, both alone and in combination with intravenous infusion of an opioid receptor agonist, just before and at the onset or reperfusion. The addition of morphine infusion to RIPC did not confer an increase in the number of patients achieving full resolution (primary measure of efficacy), but was associated with greater percentage of ST-segment resolution and lower peak TnI levels.

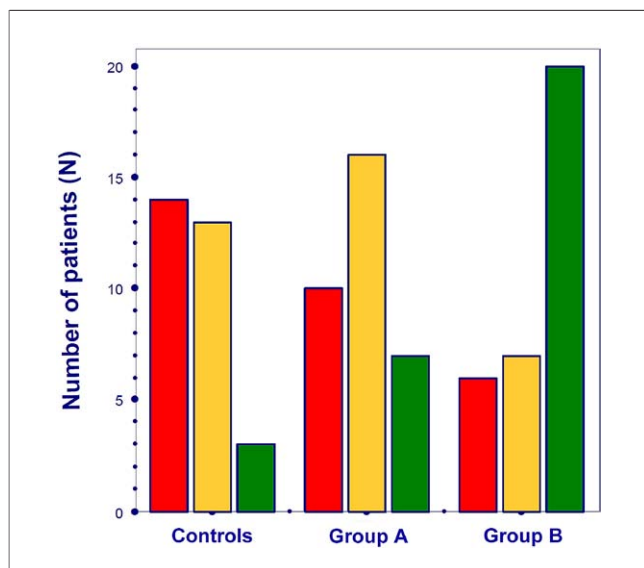


Figure 4. Distribution of Tertiles of ST-Segment Resolution per Group

Distribution of patients in tertiles of ST-segment resolution per randomization group. Most patients falling in the upper tertile of ST-segment resolution belonged to Group B ($p < 0.001$ for overall distribution difference between groups). Tertile of ST-segment resolution: **red bars** = lower tertile; **yellow bars** = middle tertile; **green bars** = higher tertile.

The protective action of ischemic post-conditioning has been known for some time, fueled by observations that gradual or intermittent, rather than sudden, reperfusion reduces the detrimental effects of ischemia-reperfusion injury (12,13). Ovize and colleagues have demonstrated that sequential cycles of reperfusion and ischemia at the time of reperfusion (by serial balloon inflations and deflations) not only leads to reduced infarct size at the immediate post-STEMI period (3), but also to a long-term improvement in left ventricular function, when compared with control patients (4). The notion of *remote* ischemic pre- and post-conditioning refers to the phenomenon of observed cardioprotection effected by short periods of ischemia applied not upon the myocardium itself, but on other organs or a limb (14–16).

Our results suggest a potentially important role of opioid action in ischemic post-conditioning. This is further supported by recent observations indicating that the cardiomyocyte-protective action of ischemic pre-conditioning is blocked by pre-treatment with the opiate receptor blocker naloxone (17). It should be duly noted, however, that the mechanisms of RIPC are not completely clear; it seems that humoral factors are involved (17–19), which need to be active during the critical early stages of reperfusion to induce protection (20,21). Alternatively, a neurogenic pathway could be activated during conditioning ischemia and induce protection in tissues (22).

One of the factors that have received attention as to their possible role in post-conditioning is mPTP. Under normal physiological conditions, the mitochondrial inner membrane is impermeable to almost all metabolites and ions, and

the mPTP is in a closed conformation. Under some stress conditions, the mPTP may open and allow the equilibration of molecules smaller than approximately 1,500 Da (6,23,24). Ischemia-reperfusion combines several conditions that can trigger mPTP opening, including matrix Ca^{2+} overload, overproduction of reactive oxygen species, depletion of adenine nucleotides, and accumulation of inorganic phosphates (6). It has also been shown that mPTP opening does not happen during ischemia, but occurs within the first 5 min of reflow following a 30-min ischemia in the isolated rat heart (25). The mPTP opening leads to destabilization and swelling of the mitochondria, with potentially lethal results for the cell, thus contributing to reperfusion injury. Post-conditioning has been shown to inhibit mPTP opening (7). Inhibition of mPTP opening has also been demonstrated to be mediated by activation of delta-opioid receptors, suggesting that opioid receptor activation-triggered post-conditioning may protect the heart by targeting the mPTP (8). It has also been demonstrated in a clinical setting that cyclosporine, an immunosuppressant drug that inhibits the opening of mPTP, attenuates lethal myocardial injury that occurs at the time of reperfusion (9). It would, thus, be a plausible assumption to make that a combination of RIPC and inhibition of mPTP opening by morphine infusion, both initiated just before reperfusion onset, could exert a cardioprotective effect.

Study limitations. The present study did not assess clinical end points (cardiovascular events and/or deaths). As a result, these observations should be considered as supportive evidence, which may be used to promote further clinical study of the potential cardioprotective role of RIPC and opioid agonists. Still, the parameters used as efficacy measures (ST-segment deviation score and peak TnI levels) are known correlates of post-myocardial-infarction outcomes and represent legitimate surrogate indicators of clinical prognosis.

Conclusions

This single-center, parallel-group, randomized study demonstrates a significant cardioprotective effect of RIPC during reperfusion, in the setting of acute STEMI. The addition of morphine infusion did not confer an improvement as far as the primary efficacy measure (full ST-segment resolution) was concerned, but was associated to greater ST-segment deviation score reduction and lower peak TnI levels. These findings are strongly suggestive of a potentially useful role of RIPC and morphine administration for the prevention of reperfusion injury in patients submitted to primary PCI.

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Key Words: remote ischemic post-conditioning ■ periconditioning ■ reperfusion injury ■ myocardial infarction ■ percutaneous coronary intervention ■ morphine.