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Impact of ischemic postconditioning with lactate–enriched blood on early inflammation after myocardial infarction

Takashi Koyama a,⁎, Hiroki Niikura b, Masaru Shibata a, Kazunori Moritani a, Megumi Shimada c, Akiyasu Baba c, Makoto Akaishi c, Hideo Mitamura a

a Cardiovascular Center, Tachikawa Hospital, 4-2-2 Nishiki-cho, Tachikawa, Tokyo 190-8531, Japan
b Cardiology Division, Ota Memorial Hospital, 455-1 Oshima-cho, Ota, Gunma 373-8585, Japan
c Cardiology Division, Kitasato Institute Hospital, 5-9-1 Shirahata, Minato-ku, Tokyo 108-8642, Japan

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A B S T R A C T

Background: Excessive early inflammation after myocardial infarction (MI) is associated with poor outcomes. However, an approach for suppressing this early inflammation has not been reported. We previously reported that postconditioning with lactate–enriched blood (PCLeB) induced excellent microcirculation recovery in patients with acute MI. We therefore tested the hypothesis that early inflammation after MI could be suppressed by PCLeB.

Methods and results: We treated 17 consecutive patients with ST-elevation MI using primary percutaneous interventional protocol within 12 h of onset. In this protocol, the duration of each brief reperfusion was prolonged from 10 to 60 s in a stepwise manner. Lactated Ringer’s solution (20–30 mL) was injected directly into the culprit coronary artery at the end of each brief reperfusion, and the balloon was quickly inflated at the site of the lesion to trap lactate within the ischemic myocardium. Each brief ischemic period lasted 60 s. After 7 cycles of balloon inflation and deflation, full reperfusion was performed; subsequently, stenting was performed. C-reactive protein (CRP) levels were measured daily and the peak values within the first 7 days post-admission were recorded. Peak CRP values were compared with those in matched control patients with acute MI treated without postconditioning. In both groups, only patients with CRP values <0.3 mg/dL on admission were included. Peak CRP values were significantly lower in the postconditioned group (control group vs. postconditioned group, 5.05 ± 4.85 vs. 1.66 ± 1.57 mg/dL; p < 0.01).

Conclusion: PCLeB may suppress early inflammation after MI.

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Introduction

Excessive early inflammation after myocardial infarction (MI) is associated with adverse left ventricular remodeling [1,2] and poor outcomes [3]. However, an approach that effectively suppresses early post-MI inflammation during/after reperfusion therapy has not been reported. We previously reported that ischemic postconditioning with lactate–enriched blood (PCLeB) induced excellent microcirculation recovery in patients with acute MI [4,5]. This has not been demonstrated in patients with acute MI treated with the original postconditioning procedures [6].

The protective effects of postconditioning are thought to result from a delayed recovery from intracellular acidosis during the early phase of reperfusion [7]. Therefore, our modified postconditioning protocol was designed based on the assumption that prolonging the delay in intracellular acidic recovery may increase the protective effects of postconditioning. The improved microcirculation recovery achieved by our approach is expected to induce a healthier healing process in the reperfused ischemic myocardium. If this is true, excessive inflammatory responses will not be evoked by reperfusion. Therefore, we tested the hypothesis that early post-MI inflammation can be suppressed by our modified postconditioning protocol.

Methods

This matched case–control study was approved by the ethics review boards of Tachikawa Hospital, Ota Memorial Hospital, and Kitasato Institute Hospital; all study patients provided informed consent.

⁎ Corresponding author at: Cardiology Division, Saitama Municipal Hospital, 2460 Mimuro, Midori-ku, Saitama City, Saitama 336-8522, Japan. Tel.: +81 48 873 4111; fax: +81 48 873 5451.
E-mail address: koyama@ame.com (T. Koyama).
Study patients

The inclusion criteria were as follows: hospital admission within 12 h of a first ST-elevation MI; a C-reactive protein (CRP) level <0.3 mg/dL upon admission; no collagen-related diseases, malignancies, or any infectious diseases; and eligibility for primary percutaneous coronary intervention (PCI). An ST-elevation MI was defined as prolonged chest pain (duration >30 min) and an ST-segment elevation ≥2 mm in ≥2 adjacent leads. Patients having an infarct-related coronary artery with a thrombolysis in Myocardial Infarction (TIMI) flow grade II or III were excluded from the study. The relevant patient characteristics are shown in Table 1.

Study protocol

We treated 17 consecutive patients who met the inclusion criteria with primary PCI using our modified postconditioning protocol (Fig. 1). In this postconditioning protocol, the duration of each brief reperfusion was prolonged from 10 to 60 s in a stepwise manner. This approach sought to prevent rapid and abrupt washout of lactate during the very early phase of reperfusion. At the end of each brief reperfusion, lactate was supplied by injecting lactated Ringer’s solution (Lactec Injection, Otsuka Pharmaceutical, Tokyo, Japan), containing 28 mM lactate, into the culprit coronary artery (20 mL for the right coronary artery, 30 mL for the left coronary artery). To trap the lactate within the ischemic myocardium, the balloon was quickly inflated with low pressure at the site of the lesion. Each brief ischemic period lasted 60 s. This approach aimed to achieve controlled reperfusion with cellular acidosis and minimal lactate washout from the cells. Lactate accumulation is generally accepted to be responsible for intracellular acidosis during ischemia. Therefore, the delay in recovery from intracellular acidosis, achieved by simple intermittent reperfusion, may be increased through this approach. After 7 cycles of balloon inflation and deflation, full reperfusion was performed; subsequently, stenting was performed. LCA, left coronary artery; and RCA, right coronary artery.

Biomarker measurement

As an indicator of inflammation, each patient’s blood CRP level was measured daily using a latex photometric immunoassay; the peak value during the first 7 days after admission was also recorded. Peak CRP values were compared with those in control patients who experienced ST-elevation MI and were successfully treated with primary PCI without postconditioning. Data from control patients, treated before 2012 in our hospitals, were also collected within 12 h of MI onset. Blood creatine kinase (CK) levels were measured daily using a latex photometric immunoassay; the peak value during the first 7 days of lactate infusion and deflation, full reperfusion was performed; subsequently, stenting was performed. LCA, left coronary artery; and RCA, right coronary artery.

Statistics

All continuous variables are reported as means ± SD. Differences between continuous variables in the 2 patient groups were assessed by a non-paired t-test. Categorical variables in the 2 groups were compared using a chi-squared test or Fisher exact test. A p-value <0.05 was considered statistically significant.

Results

The 17 study patients were successfully reperfused, achieving a grade III TIMI flow and a Blush grade of more than II. Marked myocardial staining with contrast medium and/or early visualization of venous drainage on CAG were observed in each patient, indicating unimpaired microcirculation. Figs. 2 and 3 show the final CAG views from 2 representative patients after completion of primary PCI. The figures also show the CAG view of each patient in the chronic phase (6 months after the MI), for comparison. In Fig. 2a, marked myocardial staining with contrast medium was observed in the final CAG view after completion of PCI. The myocardium was stained more densely in the acute phase than in the chronic phase (Fig. 2a and b, Videos 1 and 2). In Fig. 3a, early visualization of venous drainage was observed after PCI completion. Venous drainage was more clearly visualized in the proximal occlusion of a coronary artery.

Table 1

Characteristics of control patients and postconditioned patients.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 20)</th>
<th>PCLeB (n = 17)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>65.4 ± 11.1</td>
<td>66.6 ± 143</td>
<td>NS</td>
</tr>
<tr>
<td>Male patients, n (%)</td>
<td>15 (75.0)</td>
<td>13 (76.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Patients with diabetes mellitus, n (%)</td>
<td>5 (25.0)</td>
<td>4 (23.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Time to reperfusion, h</td>
<td>5.7 ± 3.1</td>
<td>5.8 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Peak CK value, IU/L</td>
<td>2336 ± 1143</td>
<td>2370 ± 1042</td>
<td>NS</td>
</tr>
<tr>
<td>Time from reperfusion to peak CK release, h</td>
<td>7.5 ± 4.4 (3–17 h)</td>
<td>4.5 ± 1.5 (2–6 h)</td>
<td>0.013</td>
</tr>
<tr>
<td>Proximal occlusion of a coronary artery*, n (%)</td>
<td>12 (60)</td>
<td>16 (94.1)</td>
<td>0.023</td>
</tr>
<tr>
<td>Proximal occlusion of LAD, n (%)</td>
<td>5 (25.0)</td>
<td>7 (41.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

CK, creatine kinase; LAD, left anterior descending artery; PCLeB, postconditioning with lactate-enriched blood.
* Proximal occlusion of a coronary artery includes occlusion of segments 1 and 2 of the right coronary artery, segments 6 and 7 of the left anterior descending artery, and segment 11 of the left circumflex artery.
acute phase than in the chronic phase (Fig. 3a and b, Videos 3 and 4), indicating facilitated drainage of the contrast medium through the microcirculation during the acute phase. Notably, the epicardial obtuse marginal branch of the left circumflex coronary artery appeared more spastic in the acute phase than in the chronic phase, despite the facilitated venous drainage during the acute phase.

Fig. 4 shows a comparison of peak CRP levels between the PCLeB group and the matched control group. Peak CRP levels were significantly lower in the PCLeB group than in the matched control group (control group vs. postconditioned group, 5.05 ± 4.85 vs. 1.66 ± 1.57 mg/dL; p < 0.01). This was despite similar peak CK levels between the 2 groups and inclusion of a larger proportion of patients with proximal occlusion of a coronary artery in the PCLeB group (Table 1). Notably, the time from reperfusion to peak CK release was significantly shorter in the PCLeB group (range, 2–6 h) than in the control group (range, 3–17 h; p < 0.01) (Table 1).

Discussion

Our postconditioning approach consistently resulted in excellent microcirculation recovery [4,5], and in some instances, produced coronary blood flow even better than that observed during the chronic phase. One patient showed a constricted epicardial coronary artery with facilitated venous drainage of the contrast medium (Fig. 3); this finding suggests that locally produced endogenous vasodilating substances, instead of circulating or exogenous vasodilators, are responsible for the excellent, and sometimes supra-normal, microcirculation recovery achieved.

Locally produced endogenous vasodilators such as adenosine or nitric oxide are generally believed to be involved in the reactive hyperemia that is transiently observed immediately after reperfusion of an ischemic myocardium. The reason for the transient nature of reactive hyperemia is unknown. However, the findings of the present study...
suggest that reactive hyperemia is prematurely terminated by the microvascular reperfusion injury that occurs shortly after the initiation of reactive hyperemia. If this is the case, prevention of reperfusion injury would lead to the emergence of long-lasting reactive hyperemia, unveiling the nature of the hyperemia. The exceptionally good microcirculation recovery observed in patients treated with our protocol may be explained by this mechanism; other explanations are not apparent.

Myofilaments lose their sensitivity to Ca\(^{2+}\) in the presence of intracellular acidosis. The ischemic myocardium ceases contraction by accumulating lactate to preserve adenosine triphosphate via this mechanism. Koyama et al. reported that simulated ischemia in guinea pig myocytes resulted in reperfusion-induced contracture. This was accompanied by re-sensitization of the myofilaments to Ca\(^{2+}\) resulting from reperfusion-induced lactate washout [8]. An abrupt washout of lactate by reperfusion may result in the development of hypercontracture, with re-sensitization of the myofilaments to Ca\(^{2+}\). Therefore, maintaining a high concentration of intracellular lactate during early reperfusion might prevent or minimize hypercontracture-induced myocardial damage. Our modified protocol was designed to prevent lactate extrusion from cells by maintaining high extracellular concentrations of lactate during the early reperfusion period, thereby delaying the restoration of vigorous contractile force. The strong force generated by hypercontracture compresses the microcirculation, thus obliterating coronary blood flow. The excellent microcirculation recovery achieved by our approach appears to provide good evidence that our novel postconditioning approach successfully attenuates reperfusion-induced hypercontracture.

Another finding suggestive of successful attenuation of reperfusion injury by our approach is the very early peaking of serum CK release consistently observed in the PCLeB group, irrespective of the time to reperfusion. In patients treated with PCLeB, serum CK levels peaked within 6 h of reperfusion therapy, without exception. Koyama et al. reported a biphasic relationship between the time to reperfusion and the time to peak CK release in patients who underwent successful PCI after acute MI; the latest CK release occurred in patients treated approximately 7 h after the onset of MI. Koyama et al. also showed a positive linear relationship between these 2 variables in patients treated within 7 h of onset and a negative linear relationship in patients treated more than 7 h after onset. The authors claimed that this biphasic relationship could not be explained by the traditional, simple washout mechanism of CK, but was explained after considering the significant contribution of cell death caused by reperfusion injury on early CK release [9]. In the present study, this biphasic relationship between the time to reperfusion and the time to peak CK release was not observed in patients treated with PCLeB. This may be regarded as additional evidence for the successful attenuation of reperfusion injury by PCLeB because the very early peaking of CK release, irrespective of time to reperfusion, can be explained simply by the traditional washout CK mechanism [10].

**Fig. 3.** Coronary angiography (CAG) of a second representative patient. (a) Panel 0: CAG before percutaneous coronary intervention (PCI), showing total occlusion of the left circumflex artery. Panel 1: The final CAG view after completion of stenting. Panel 2: Late phase of the final CAG view; the obtuse marginal vein is clearly visualized (arrows). (b) CAG of the same patient 6 months later. Panel 1: CAG in the early phase. Panel 2: The late phase of the same CAG view. The obtuse marginal vein looks blurred and is not as clearly visualized as that in the acute phase, whereas the obtuse marginal artery is clearly dilated (arrows), as compared with that during the acute phase.

**Fig. 4.** Comparison of peak C-reactive protein levels between control patients and postconditioned patients. CRP, C-reactive protein; PCLeB, postconditioning with lactate-enriched blood.
In our approach, postconditioning was prioritized over thrombosuction. The original postconditioning protocol was intended to prevent the formation of distal emboli by employing a direct stenting procedure [6]. However, direct stenting is not always safe and is therefore not recommended for all patients with acute MI. Accordingly, pharmacological postconditioning is currently being explored in an attempt to avoid direct stenting. Unfortunately, the effectiveness of pharmacological postconditioning has not been proven in large-scale clinical trials. Therefore, to avoid direct stenting, it is necessary to perform either postconditioning or thrombosuction for reducing infarct size. We chose to prioritize postconditioning over thrombosuction because we believe that the amount of ischemic myocardium facing a risk of lethal reperfusion injury was far larger than the amount of ischemic myocardium that could not be salvaged because of the distal emboli that could not be retrieved by thrombosuction after the postconditioning procedures. In addition, in many cases, thrombosuction may not result in complete removal of all of the embolic materials, even when this procedure is undertaken first. Given the excellent microcirculation recovery achieved by our approach, this postconditioning protocol appears to surpass thrombosuction in terms of the restoration of microcirculatory function. The basic concept of our postconditioning protocol is the maintenance of endogenous lactate inside the cells, thereby preventing rapid restoration of contractile force upon reperfusion. If thrombosuction is undertaken first and uncontrollable coronary flow recovery ensues, a considerable amount of the lactate accumulated in the ischemic myocardium would be washed away before the postconditioning procedures, and vigorous myocardial contractile force would be quickly restored. We believe that this sequence is hazardous to the reperfused myocardium. The present postconditioning protocol prevented this sequence by employing lactate as an inherent contractile activity blocker; this protocol was prioritized over thrombosuction.

In the present study, peak CRP levels were significantly lower in the PCLeB group than in the matched control group. Because the mean peak CRK value in the PCLeB group was relatively low, only patients with relatively small infarct sizes, estimated by the basis of peak CK values, were included in the matched control group. In fact, significantly fewer patients had proximal occlusion of a coronary artery in the matched control group than in the PCLeB group. Nonetheless, a significantly reduced increase in CRP levels was observed in the PCLeB groups. Immediately after MI, CRP levels are positively related to peak CK levels [2]; therefore, the peak CRP levels in the matched control group should be lower than those in patients with acute MI among the general population. In fact, the peak CRP levels in the control patients were substantially lower than those reported in the literature [1–3]. Accordingly, patients treated with the modified postconditioning protocol were disadvantageously compared with the control patients in terms of the severity of their early inflammation after MI. Thus, the differences in the peak CRP levels between the 2 groups in this study were probably underestimated. Therefore, despite the small and non-randomized nature of this study, the results obtained here should reflect the protective effect of this new treatment strategy. This statement may be supported by the other, aforementioned findings in the present study, suggestive of successful attenuation of reperfusion injury.

The present study had some limitations. First, it was a small-scale study involving a limited number of patients. Despite the small-scale nature of the study, the results appear to be promising. However, additional investigations involving a larger number of patients are required to elucidate the true value of this new treatment strategy. Second, it was not a randomized controlled trial involving prospectively enrolled patients. As physicians, we found it difficult not to treat patients experiencing severe chest pain with our novel approach. We were aware that patients with acute MI would achieve rapid relief with our approach in terms of rapid resolution of their chest pain and scant serious reperfusion arrhythmias, as previously reported [4,5]. This would result in safer subsequent procedures, along with maintaining the patient in a better condition, both physically and mentally, during and after the procedures. Furthermore, we believed that the consistently observed, excellent microcirculation recovery achieved by our approach might improve the long-term outcomes of the patients. Thus, we could not justify, performing a randomized control trial, and therefore, conducted a matched case-control study, instead. Third, we did not compare the effects of our modified postconditioning protocol with those of the original postconditioning protocol without lactate manipulation. The excellent microcirculation recovery achieved by our approach has never been reported with the original postconditioning procedures [6]. Moreover, the original postconditioning protocol reportedly does not prevent the no-reflow phenomenon in animal experiments [11]. Our approach has consistently shown excellent microcirculation recovery and occasionally, extremely good, microcirculation recovery, which is in stark contrast to the no-reflow phenomenon. Given the recent controversy regarding the protective effects of the original postconditioning procedures [12,13], the benefit of the addition of lactate to the heart during the intermittent reperfusion postconditioning seems apparent. In terms of the relevance of violent environmental changes occurring inside the ischemic myocardium upon reperfusion, the supplemental addition of lactate to the heart during the intermittent reperfusion might be quite rational and possibly crucial for myocardial protection. Given the safety aspects, especially the absence of pharmacological side effects of lactated Ringer’s solution, we believe that injecting lactated Ringer’s solution during the intermittent reperfusion of the postconditioning procedures is worthwhile, even in the absence of any direct comparative evidence.

In conclusion, ischemic postconditioning with lactate-enriched blood may suppress early inflammation after MI. However, further investigations involving a larger number of patients are needed to examine the true value of this new approach for treating patient with acute MI.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijicme.2014.02.002.

Disclosures
None.

References