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Depletion of Antioxidants Is Associated with No-Reflow Phenomenon in Acute Myocardial Infarction

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Summary

Background: No-reflow phenomenon is observed in approximately one-third of patients after percutaneous coronary intervention (PCI) for acute myocardial infarction (AMI), and is associated with poor functional and clinical outcomes. On the other hand, the formation of free radicals in vasculature exerts deleterious effects on coronary microcirculation.

Hypothesis: We hypothesized that redox state in coronary circulation may play a crucial role in no-reflow phenomenon in AMI.

Methods: Consecutive 26 patients with first AMI who underwent primary PCI < 24 h after onset were enrolled. Before PCI, blood samples were obtained from coronary sinus to measure plasma or serum antioxidative vitamins (vitamin C, vitamin E, and beta-carotene) and antioxidative enzymes (extracellular glutathione peroxidase [GPX], superoxide dismutase, and catalase). After PCI, the corrected Thrombolysis In Myocardial Infarction (TIMI) frame count (CTFC) was measured in the target vessel. Patients with TIMI ≤ 2 flow despite an optimal PCI result were designated as no-reflow group (Group NR, n = 6) and the others as reflow group (Group R, n = 20).

Results: Levels of vitamin C, vitamin E, and GPX before PCI were significantly lower in Group NR than in Group R. The CTFC correlated inversely with levels of vitamin C, vitamin E, and GPX ($p < 0.05$).

Conclusions: Depletion of antioxidants is associated with no-reflow phenomenon in AMI. These findings strongly suggest that the redox state in coronary circulation plays an important role in the pathogenesis of no-reflow phenomenon.

Key words: oxidative stress, reperfusion, percutaneous coronary intervention, coronary circulation

Introduction

Coronary reperfusion therapy is widely performed to preserve left ventricular function and improve the prognosis in patients with acute myocardial infarction (AMI). However, successful recanalization of the occluded epicardial coronary artery does not necessarily lead to myocardial salvage, because of the "no-reflow'" phenomenon that is observed in approximately one-third of patients with reperfused MI. No-reflow phenomenon is defined as inadequate myocardial perfusion without angiographic evidence of mechanical obstruction, and the occurrence of this phenomenon in patients with no-reflow has been reported to have poor functional recovery and prognosis.[1] It has become evident that abnormalities of microvascular circulation cause the no-reflow phenomenon; however, their exact mechanisms and pathogenesis are not determined.

Reactive oxygen species (ROS) have profound impacts on vascular function. For example, ROS not only inactivate endothelium-derived nitric oxide, but also induce directly endothelial injury. Furthermore, a successful coronary reperfusion results in marked enhancement in the release of ROS from inflammatory cells accumulated in the infarcted area. Therefore, there is a possibility that the oxidative stress plays an important role in the no-reflow phenomenon.[2] We hypothesized that depletion of antioxidants may lead to the no-reflow phenomenon in patients with AMI. To test this hypothesis, we measured the serum levels of antioxidative vitamins and enzymes in patients with AMI before reperfusion.

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Methods

Study Patients

The study group consisted of consecutive 26 patients with first AMI who were successfully recanalized with primary percutaneous coronary intervention (PCI) (< 50% diameter stenosis after PCI) within 24 h after onset of symptoms. Patients with primary myocardial disease, unstable hemodynamic state, inflammatory disease, and malignancy were excluded. The study protocol was approved by the ethics committee of Kobe University Hospital. Informed consent was obtained from all patients before undergoing cardiac catheterization.

Cardiac Catheterization and Coronary Intervention

After intravenous injection of 2000 U of heparin, left ventriculography and coronary angiography were performed using the standard femoral approach. Nitroglycerin (0.2 mg) was administered via intracoronary injection as angiography was performed. After diagnostic angiography, 8000 U of heparin was injected intravenously. Percutaneous coronary intervention was performed in the usual manner via femoral approach with standard techniques. Neither anticoagulants nor thrombolytic agents were administered. Quantitative coronary angiography (QCA) was performed using an autoedge detection method with a commercially available system (CCIP-310, Cathex Company, Tokyo, Japan).

Assessment of Coronary Flow

Thrombolysis In Myocardial Infarction (TIMI) flow grade was assessed as previously described.[3] Patients with TIMI ≤ 2 flow despite an optimal PCI result were designated as no-reflow group (Group NR) and the others as reflow group (Group R). Immediately after PCI, the corrected TIMI frame count (CTFC) was measured in the infarct-related artery as previously described.[4] Cinefilm was recorded at a speed of 30 frames/s.

Blood Sampling and Measurement of Antioxidants

After diagnostic coronary angiography, blood samples were obtained from the coronary sinus just before PCI. Serum vitamin C, vitamin E, and beta-carotene were measured by high-performance liquid chromatography. Plasma glutathione peroxidase (GPX) was analyzed by enzyme immunoassay (Bioxytech®, Oxis International, Portland, Ore., USA). Serum superoxide dismutase (SOD) was measured using the nitro blue tetrazolium (NBT) method, and serum catalase was measured by colorimetry.

Statistical Analysis

Continuous values are expressed as mean ± standard deviation (SD). Comparisons of continuous variables were performed using the unpaired *t*-test or Mann-Whitney U test wherever applicable. Clinical variables were compared by chi-square test. Differences were considered significant at $p < 0.05$.

Results

Patient Characteristics and Clinical Results

Baseline clinical characteristics and patient profiles are shown in Table I. There was no significant difference between the two groups with regard to age, gender, incidence of coronary risk factors, elapsed time to recanalization, peak creatine phosphokinase, and hemodynamic data before PCI.

Angiographic results are shown in Table II. There was no difference in culprit location, number of diseased vessels,

TABLE I Clinical characteristics and hemodynamic data

	Group NR (n = 6)	Group R (n = 20)	p Value
Age	69 ± 12	65 ± 9	NS
Male	6/6	13/20	NS
Coronary risk factors			
Hypertension	1/6	8/20	NS
Hyperlipidemia	2/6	8/20	NS
Diabetes mellitus	4/6	2/20	NS
Smoking	5/6	12/20	NS
Elapsed time to recanalization (h)	11.7 ± 10.1	10.3 ± 7.2	NS
Peak creatine kinase (U/l)	4221 ± 1247	3186 ± 438	NS
Cardiac catheterization data			
Cardiac index (l/min/m^2)	2.8 ± 0.7	3.0 ± 0.6	NS
Pulmonary capillary wedge pressure (mmHg)	15 ± 5	11 ± 5	NS
Left ventricular end-diastolic pressure (mmHg)	23 ± 10	18 ± 9	NS
Mean aortic pressure (mmHg)	97 ± 16	101 ± 21	NS
Left ventricular ejection fraction (%)	50.2 ± 10.4	46.8 ± 9.3	NS

TABLE II Angiographic data

	Group NR (n = 6)	Group R (n = 20)	p Value
CAG findings			
Culprit location (LAD/LCx/RCA)	3/0/3	10/1/9	NS
Number of diseased vessels (1VD/2VD/3VD)	1/2/3	9/6/4	NS
TIMI grade before PCI (0/1/2/3)	3/1/0/2	12/1/3/4	NS
Collateral grade (0/1/2/3)	3/1/2/0	6/9/5/0	NS
Stenting	6/6	17/20	NS
Corrected TIMI frame count after PCI	46.3 ± 9.3	25.4 ± 6.5	< 0.01
QCA data			
% Diameter stenosis before PCI (%)	92.5 ± 11.5	93.9 ± 11.2	NS
% Diameter stenosis after PCI (%)	24.0 ± 14.7	15.1 ± 12.2	NS
Minimal lumen diameter (mm)	0.15 ± 0.38	0.15 ± 0.26	NS
Reference diameter (mm)	3.32 ± 0.50	2.91 ± 0.67	NS

Abbreviations: CAG = coronary angiography, LAD = left anterior descending coronary artery, LCx = left circumflex coronary artery, PCI = percutaneous coronary intervention, QCA = quantitative coronary angiography, RCA = right coronary artery, TIMI = Thrombolysis In Myocardial Infarction.

TIMI grade before PCI, collateral grade, or use of coronary stents between the two groups. Primary PCI was successfully performed in all patients. After primary PCI, TIMI 2 flow was observed in 6 patients (Group NR) and TIMI 3 flow in 20 patients (Group R). No patient had a TIMI 0 or 1 flow. The CTFC was significantly higher in Group NR than in Group R ($p < 0.01$). There was no difference in the QCA parameters between the two groups.

Levels of Antioxidants

As shown in Figure 1, levels of vitamin C (0.08 ± 0.20 μg/ml vs. 1.05 ± 1.25 μg/ml, $p < 0.05$), vitamin E (9.45 ± 2.10 μg/ml vs. 12.71 ± 3.39 μg/ml, $p < 0.05$), and GPX (26.52 ± 7.30 μg/ml vs. 41.47 ± 19.16 μg/ml, $p < 0.05$) were significantly lower in Group NR than in Group R before PCI, while there was no significant difference in levels of beta-carotene, SOD, or catalase (Fig. 1). It was of great interest that CTFC correlated inversely with levels of vitamin C, vitamin E, and GPX ($r = -0.415$, $p < 0.05$; $r = -0.482$, $p < 0.05$; and $r = 0.434$, $p < 0.05$, respectively) as shown in Figure 2. There was no significant correlation between CTFC and levels of beta-carotene, SOD, or catalase (Fig. 2).

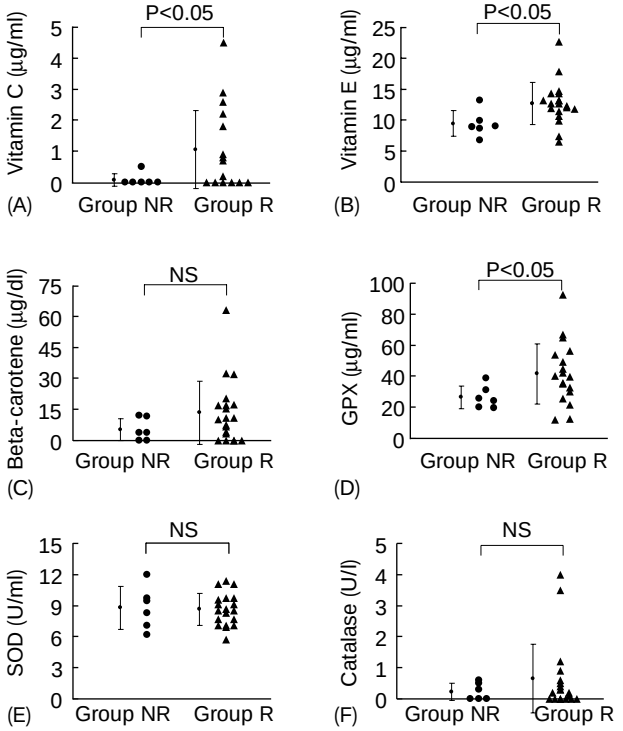

FIG. 1 Levels of vitamin C (A), vitamin E (B), beta-carotene (C), glutathione peroxidase (GPX) (D), superoxide dismutase (SOD) (E), and catalase (F) before reperfusion in 6 patients with TIMI ≤ 2 flow (Group NR) and 20 patients with TIMI 3 flow (Group R) after reperfusion.

Discussion

In the present study, we have demonstrated that the levels of several antioxidants such as vitamin C, vitamin E, and GPX before PCI were markedly reduced in patients with no-reflow phenomenon. Concomitantly, the levels of these antioxidants also correlated inversely with CTFC. Some pathophysiologic situations may affect the levels of antioxidants such as hypertension, smoking status, diabetes mellitus, and hyperlipidemia; however, baseline clinical and angiographic characteristics were not different between the groups. These findings strongly suggest that oxidative stress plays a critical role in the no-reflow phenomenon.

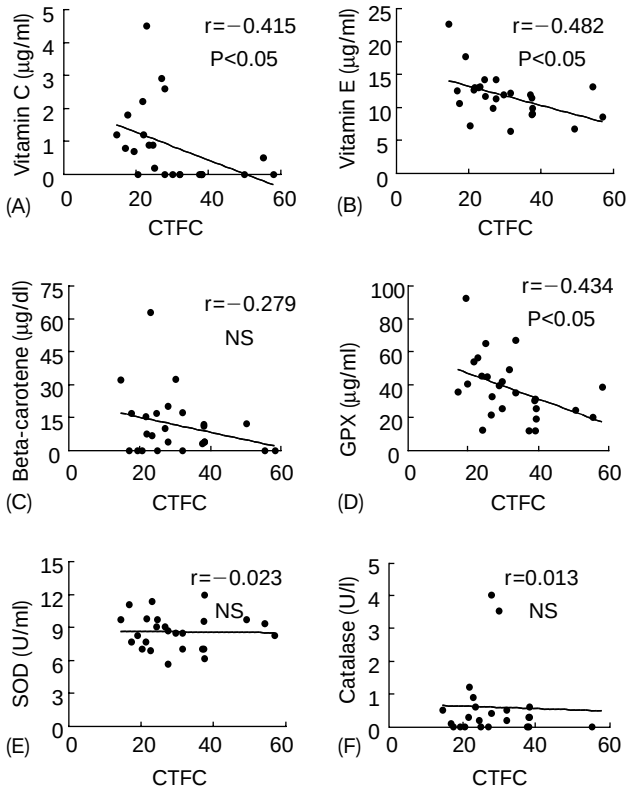

FIG. 2 The relation between corrected TIMI frame count (CTFC) and levels of vitamin C (A), vitamin E (B), beta-carotene (C), glutathione peroxidase (GPX) (D), superoxide dismutase (SOD) (E), and catalase (F) before reperfusion. The CTFC correlated inversely with levels of vitamin C, vitamin E, and GPX. There was no significant correlation of levels of beta-carotene, SOD, or catalase with CTFC.

The abnormalities of microvascular circulation cause the no-reflow phenomenon. So far, several mechanisms of microvascular dysfunction are proposed, including microvascular obstruction by plugging of leukocytes or platelets, microembolization of plaque debris, vasospasm, and reperfusion injury.[5] The subsequent release of oxygen-free radicals from the plugged leukocytes will cause further injury to the capillary endothelium.[2] Moreover, the reintroduction of molecular oxygen into ischemic tissue upon reperfusion leads to excessive formation of oxygen-free radicals. Thus, the oxidative stress has been implicated as a mediator of coronary arterial dysfunction that may lead not only to the no-reflow phenomenon but also to cardiac dysfunction.[2, 6] Our findings in the present study support the role of oxidative stress in this process.

The cellular protection against oxidative stress is categorized into an enzymatic or nonenzymatic defense system. Pandey *et al.* reported a remarkable increase in platelet xanthine oxidase activity and a rise in the level of malondialdehyde with concomitant decrease in free radical scavenging enzymes in ischemia/reperfusion.[7] The PCI procedure would lead to the generation of free radicals that may overwhelm the tissue antioxidant defense capacity. Thus, it is suggested that the localized enzymatic defense response system plays a crucial role in countering the sudden increases in oxygen free radicals, and a change of these enzymes could significantly affect the prognosis.

The enzymatic defense systems have several isoforms that differ in their tissue distribution, subcellular localization, and the cofactors required for catalytic activity. There are three isoforms of SOD, that is, Cu/Zn SOD, Mn SOD, and extracellular SOD (EC-SOD). Cu/Zn SOD is located in the cytosol, while Mn SOD is primarily in the mitochondria. Glutathione peroxidase also has several isoforms such as cytosolic GPX (GPX-1), phospholipid GPX, gastrointestinal GPX, and extracellular GPX. In the present investigation, the activities of antioxidative enzymes in blood were measured; therefore, they reflect mainly the activities of extracellular isoforms. Since EC-SOD has a heparin-binding site, its activity is mainly associated at the surface of the endothelium. It is also reported that EC-SOD activity is also expressed in macrophages in the atherosclerotic vessels. These explain, in part, the very few changes in SOD in our study. On the other hand, catalase is ubiquitously located in the cytosol of all cell types; therefore, its enzymatic activity in serum mainly reflects the leakage from cells such as erythrocytes. Our findings indicate its insignificant role in the situation of no-reflow phenomenon.

Glutathione peroxidase-1 is reported to play a crucial role in myocardial protection from ischemic reperfusion injury. Its knockout mice subjected to myocardial ischemia/reperfusion have a poor functional recovery,[8] while its cardiac-specific overexpression induced resistance to ischemia-reperfusion injury and improved contractility after reperfusion.[9] Our present study shows a significant reduction in extracellular GPX before PCI in patients with no-reflow phenomenon, which furthermore correlated inversely with CTFC. Taken together, it is suggested that the cytosolic as well as the extracellular isoform of GPX play a prominent role in cardiac protection against oxidative stress in ischemic/reperfusion.

Nonenzymatic defenses such as vitamin C, vitamin E, beta-carotene, and flavinoids are also equipped for carrying out the protective function against oxidative stress. It has been shown in previous studies that exogenous administration of vitamin E analogue or dietary supplementation of alpha-tocopherol improves ischemia-reperfusion injury and the myocardial mechanical function. This effect can be attributable to the detoxification of free radicals.[10, 11] Our data show a significant reduction in the levels of vitamin C and E before PCI in patients with no-reflow phenomenon, and they correlate inversely with CTFC, whereas beta-carotene was not reduced. It is consistent with earlier studies of Carrasquedo *et al.*,[12] in which they found that a change in beta-carotene was not associated with lower creatine phosphokinase (CPK) release and AMI extension.

Various methods have been proposed to assess no-reflow phenomenon using coronary angiograms,[13] myocardial contrast echocardiography,[1] Doppler flow wire study,[14] and scintigraphy.[15] In the present study, CTFC was applied to evaluate this phenomenon. This method has been used accurately in previous studies to identify the no-reflow phenomenon on

coronary angiograms.[4] Furthermore, CTFC has been recently reported as a predictor of clinical outcome and functional recovery in patients with AMI after reperfusion therapy.[16, 17]

Study Limitations

Some of the possible limitations of our study are outlined: (1) Antioxidative enzymes act and detoxify free radicals intracellularly as well as extracellularly; however, in our study, we have measured only the levels of extracellular isoforms. Although their intracellular levels may have provided precise information, it is impossible to assess the redox state in vascular cells in coronary arteries in patients with AMI. Furthermore, it is beyond the scope and goal of our present clinical study. (2) We have not evaluated the generation of free radicals in patients in our study. The production of oxygen-free radicals varies dramatically during the ischemia/reperfusion process and is very unstable to measure in the clinical settings. (3) The number of patients, especially in the group with decreased TIMI flow after reperfusion, was small. However, it is very difficult to obtain informed consent from a large number of patients in an emergent situation such as our investigation.

Conclusion

Patients with no-reflow phenomenon had reduced blood levels of antioxidants before PCI and the levels of these antioxidants correlated inversely with CTFC, suggesting that depletion of antioxidants is directly associated with no-reflow phenomenon in patients with AMI. Therapeutic interventions to prevent no-reflow are not fully established. Further investigation is necessary to determine whether administration of antioxidants prevents no-reflow phenomenon in patients with AMI.

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