Effect of Short-Term Low-Intensity Exercise Training on Association of Oxygen Free Radicals and Nitric Oxide Production in Patients with Acute Myocardial Infarction

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Abstract

Moderate-to-high intensity of exercise training within 2 to 3 months decreases oxygen free radicals (reactive oxygen species, ROS) and increases nitric oxide (NO) in outpatients with myocardial infarction. There is no data about the association of ROS and NO after short-term low-intensity exercise training within 5 days in patients hospitalized with acute myocardial infarction (AMI). A total of 32 male patients with AMI were randomized into two groups: 15 patients with short-term low-intensity exercise training within 5 days formed the training group and 17 patients without such exercise training formed the control group. All patients performed exercise treadmill test with modified Bruce protocol before and after the study. F2-isoprostane and NO concentration of the training group increased slightly after modified Bruce exercise treadmill test. Compared with the control group, NO of the training group was also slightly higher. Baseline NO and uric acid were negative predictor variables for F2-isoprostane in all patients hospitalized with AMI, and triglyceride was a positive predictor variable. After the study, physical capacity of the training group was higher; but heart rate and systolic blood pressure were lower significantly. This study showed that short-term low-intensity exercise training for patients hospitalized with AMI did not change ROS and NO productions, but it improved physical capacity and lowered heart rate and systolic blood pressure. NO was negative predictor variable for F2-isoprostane in controlling ROS changes in dynamic compensation mechanism.

Keywords

- acute coronary syndrome
- atherosclerosis
- cardiovascular risk factors
- myocardial infarction
- oxidative stress
- nitric oxide
- cardiac markers
- exercise

Oxygen, although is highly required for living, can be toxic because it can be easily changed into oxygen free radicals (reactive oxygen species, ROS) and generates oxidative stress.^{1–5} This oxidative stress will cause endothelial lesion and will be responded by endothelial cells through nitric oxide (NO) response and immunological response pathway.^{6–8} The immunological response begins by increasing cytokines production, activated coagulation factors, and reactivation of platelet in acute thrombotic process. However,

acute thrombotic process, which is an initial part of protective mechanism to fix the lesion, often change to become continuous process because of abundance oxidative stress, and then generates various levels of acute coronary syndromes.^{3,5} Several studies showed that acute exercise on treadmill test with high intensity Bruce protocol increased oxidative stress because of ROS.^{9,10} On the contrary, acute exercise also increased production of NO because of the increment effect of shear stress on endothelial cells.^{6,9–15} But, regular exercise

training with moderate-to-high intensity—attenuated oxidative stress in outpatients with recent myocardial infarction after 2 to 3 months of such exercise training.^{16–18}

Production of ROS in well-trained patients was lower than in sedentary patients, meanwhile, NO was higher.^{19–23} It also had been shown that short-term low-intensity exercise training within 5 days could rapidly elevate internal antioxidants in the diaphragm muscle of rats, accompanied by a reduction in contraction-induced lipid peroxidation or ROS production, but generated high NO consistently.²⁴ Effect of short-term low-intensity exercise training within 5 days on ROS and NO concentration in patients hospitalized with acute myocardial infarction (AMI) has not been demonstrated.

The aim of this study was to evaluate the effects of short-term low-intensity exercise training on various clinical parameters as well as molecular parameters, such as plasma F2-isoprostane (an accurate and specific marker of ROS)^{25–27} and plasma nitrite and nitrate production (the marker of NO)^{28,29} in patients with AMI.

Methods

Subjects

There were 32 male patients (age range, 41–65 years) hospitalized with AMI and randomized to 15 patients of the training group with short-term low-intensity exercise training within 5 days and 17 patients of the control group without any physical activities but bed rest in intermediate ward. To control the effect of confounding variables that could affect F2-isoprostane and NO production or influence the sessions of exercise treadmill test and short-term low-intensity exercise training sessions, patients with any of the following conditions were excluded: recurrent angina, heart failure, atrial fibrillation, atrioventricular blocks, ventricular tachyarrhythmias, valvular heart disease, chronic obstructive pulmonary disease, and renal, hepatic or hematologic dysfunction. During hospitalized in intensive care unit and intermediate ward, patients, that received anticholesterol drugs, antidiabetic drugs, and antioxidant vitamins were also excluded.

Study Protocol

In our hospital, after hospitalized in the intensive care unit within 2 to 3 days, all patients were transferred into the intermediate ward for 5 to 7 days. They underwent exercise treadmill test with modified Bruce protocol in the first day of the study and 5 days later before returning home. About 10 mL venous blood was aspirated before and after exercise treadmill test in the morning for measuring F2-isoprostane level with competitive enzyme-linked immunoassay (ELISA kit, Oxford Biomedical Research Inc., Rochester Hills, MI)^{25–27} and NO were measured with Griess reagent and spectrophotometry, as previously described.^{28,29} Treatment with any cardiovascular medications was discontinued for at least 24 hours and fasting at least 12 hours at night before exercise treadmill test in the morning.

Under closed supervision, 15 patients of the training group were expected to perform short-term low-intensity exercise training within 5 days in hospital—defined as walking on

treadmill property for 20 minutes with intensity of 20 to 40% $\text{Vo}_{2\text{max}}$ (similar to 4–5 Mets or walking slowly with the intensity of 4–5 km/h and target heart rate of 100–120/min), in addition to 5 minutes for warming up and 5 minutes for cooling down during each session. In our institution, such exercise training has been a part of standard cardiac rehabilitation program Phase 1 for patients hospitalized with AMI.⁶

The protocol was approved by the Institutional Ethics Committee of Harapan Kita Hospital, National Cardiovascular Center, and informed consent was obtained from all subjects.

Statistical Analyses

Comparing the baseline characteristic data between F2-isoprostane and NO concentration of the training group and the control group, we used either unpaired *t*-test or Mann-Whitney *U* test, according to the type of data (numeric data with normal distribution or abnormal distribution) with SPSS 13.0 for windows (IBM, Chicago, IL). Comparing the level of F2-isoprostane before and after exercise treadmill test, we used paired *t*-test or Wilcoxon test; and so with NO. For correlation and regression between variables, we used univariate Pearson correlation and multivariate linear regression analyses.³⁰ Numeric data were expressed in mean $\pm$ standard deviation. The values $p < 0.05$ were statistically significant and $p < 0.01$ were highly significant.

Results

Baseline Characteristics

In the initial study, there were no differences of baseline characteristic data between the training group and the control group in regard with age, body weight, body height, infarction, troponin T, creatine kinase MB-type (CKMB), hemoglobin, lipids, glucose, ureum, creatinine, uric acids, and medications ($\blacktriangleright$ Table 1).

F2-Isoprostane and NO Production in Patients Hospitalized with AMI

Of the total 32 patients with AMI, 1 patient of the control group had no data of baseline F2-isoprostane and NO concentration. Baseline F2-isoprostane of all 31 patients with AMI (15 patients of the training group and 16 patients of the control group only) increased significantly until 2392.6 ± 2042.5 pg/mL (range, 150.0–8960.0 pg/mL); meanwhile, the F2-isoprostane concentration of healthy persons was only 35.0 ± 6.0 pg/mL.^{31,32} On exercise treadmill test with modified Bruce protocol, F2-isoprostane concentration also increased until 1732.6 ± 1149.6 pg/mL (range, 150.0–5780.0 pg/mL). There were no differences of baseline F2-isoprostane concentration before and after acute exercise on treadmill test in all 31 patients ($p = 0.170$).

Baseline NO production was higher in all 31 patients with AMI ($39.6\text{--}200.8$ $\mu\text{mol/L}$); meanwhile, normal value of NO in healthy persons was only 32.0 ± 4.9 $\mu\text{mol/L}$.^{6,33} On exercise treadmill test with modified Bruce protocol, NO concentration also increased to 110.7 ± 58.5 $\mu\text{mol/L}$ (range, 61.0–348.8 $\mu\text{mol/L}$), but there were no differences of baseline NO

Table 1 Baseline characteristics of patients with acute myocardial infarction

Baseline	Training group	Control group	<i>p</i>
Characteristics	<i>n</i> = 15	<i>n</i> = 17	
Age, y, mean ± SD	53.5 ± 7.3	53.1 ± 8.7	0.887
Range	41–66	37–65	
Body weight, kg, mean ± SD	69.1 ± 7.8	66.9 ± 11.3	0.546
Body height, cm, mean ± SD	164.7 ± 5.8	165.4 ± 6.6	0.739
Body mass index, kg/m ² , mean ± SD	25.5 ± 2.7	24.4 ± 3.3	0.312
Infarction			
Non-Q	2	3	0.539
Anterior-Q	9	11	
Inferior-posterior-Q	4	3	
Troponin T, ng/mL, mean ± SD	1.0 ± 0.9	0.6 ± 0.7	0.446
CKMB, u/L, mean ± SD	134.1 ± 111.9	104.2 ± 96.5	0.424
Medications			
Thrombolytics	8	4	0.087
Nitrates	14	15	0.928
Beta blockers	8	7	0.498
Ca blockers	4	2	0.289
ACE inhibitors	11	14	0.544
Aspirin	15	17	1.000
Hemoglobin, g/dL, mean ± SD	14.5 ± 1.5	14.3 ± 1.5	0.827
Total cholesterol, mg/dL, mean ± SD	204.8 ± 53.0	181.9 ± 32.2	0.144
LDL cholesterol, mg/dL, mean ± SD	138.9 ± 40.1	119.9 ± 28.8	0.133
HDL cholesterol, mg/dL, mean ± SD	33.1 ± 7.8	40.6 ± 14.1	0.077
Triglyceride, mg/dL, mean ± SD	143.6 ± 58.8	145.5 ± 73.1	0.936
Plasma glucose, mg/dL, mean ± SD	176.9 ± 70.6	138.5 ± 49.0	0.081
Ureum, mg/dL, mean ± SD	24.5 ± 9.4	26.5 ± 9.7	0.558
Creatinine, mg/dL, mean ± SD	1.1 ± 0.3	0.9 ± 0.3	0.129
Uric acid, mg/dL, mean ± SD	6.6 ± 1.1	5.9 ± 1.4	0.103

Abbreviations: ACE, angiotensin-converting enzyme; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

concentration before and after exercise treadmill test in all 31 patients ($p = 0.157$).

All of independent variables correlated with dependent variable (F2-isoprostane concentration at baseline) with $p < 0.25$ were entered to multivariate regression analyses, and the results showed that baseline NO and uric acid were obviously significant negative predictor variables for F2-isoprostane concentration; meanwhile, triglyceride was as a positive predictor variable (►Table 2).

F2-Isoprostane and NO Production in the Training Group and the Control Group

F2-isoprostane and NO production did not differ significantly between the training group and the control group, before and after exercise treadmill test (►Fig. 1). F2-isoprostane production in the training group increased slightly on exercise treadmill test, from 2197.1 ± 1312.6 pg/mL to

2312.1 ± 1322.0 pg/mL, but not significant ($p > 0.05$). NO production in the training group also increased slightly on exercise treadmill test, from 110.4 ± 42.1 μmol/L to 125.2 ± 72.7 μmol/L ($p > 0.05$). Compared with the control group, NO production in the training group was higher, but not significant (125.2 ± 72.7 μmol/L vs. 110.3 ± 27.8 μmol/L).

Hemodynamic Responses

Short-term low-intensity exercise training led to increase the duration of modified Bruce exercise treadmill test and physical capacity in the training group significantly (►Fig. 2). After completing exercise training sessions in 5 days, the duration of modified Bruce exercise treadmill test and physical capacity increased significantly (from 7.8 ± 2.3 to 10.3 ± 2.3 min, $p < 0.001$; and from 4.6 ± 1.3 to 6.1 ± 1.9 Mets, $p < 0.001$, respectively). In the control group, the duration of modified

Table 2 The predictor variables for F2-isoprostane concentration at baseline in all 31 patients with acute myocardial infarction

Variables	α	β	SE	p	95% CI
Constanta	6223.97		1935.677	0.003	2252.289–10195.650
Triglyceride		12.152	5.153	0.026	1.579–22.725
Uric acid		–549.192	246.078	0.034	–1054.103–44.282
Nitric oxide		–21.808	9.223	0.025	–40.732–2.884

Abbreviations: α , constanta/intercept; β , regression coefficient; CI, confidence interval; p , probability; SE, standard error.

Note: Independent variables that correlated with dependent variable (F2-isoprostane concentration at baseline) with $p < 0.25$ entered to multivariate regression analyses, and the results showed that triglyceride, uric acid, and nitric oxide at baseline were significant predictor variables for F2-isoprostane.

Bruce exercise treadmill test and the physical capacity were not different before and after study (respectively, from 6.5 ± 3.4 to 8.1 ± 3.0 min; and from 4.0 ± 1.6 to 5.0 ± 2.0 Mets, $p > 0.05$).

—Fig. 3 showed that the maximal heart rate on modified Bruce exercise treadmill test in the training group was lower than in the control group (115.3 ± 15.8 vs. 126.4 ± 13.3 per min, $p = 0.038$). Short-term low-intensity exercise training could improve the maximal heart rate on modified Bruce exercise treadmill test significantly (119.0 ± 14.0 vs. 115.3 ± 15.8 per min, $p = 0.037$). In the control group, the maximal heart rate on modified Bruce exercise treadmill test was not different before and after study (126.1 ± 15.9 and 126.4 ± 13.3 per min).

Short-term low-intensity exercise training led to significantly lower systolic blood pressure on modified Bruce treadmill test in the training group (from 137.7 ± 22.2 to 132.3 ± 19.5 mm Hg, $p = 0.024$). Compared with the control group, systolic blood pressure on modified Bruce exercise treadmill test was also lower in the training group (132.3 ± 19.5 vs. 141.8 ± 21.9 mm Hg). There were no significant side effects or any complications during exercise treadmill test with modified Bruce protocol or short-term low-intensity exercise training.

Discussion

This study showed that short-term low-intensity exercise training did not change the production of ROS in patients hospitalized with AMI. F2-isoprostane production of the training group increased slightly but not different with the control group, even before or after exercise treadmill test with modified Bruce protocol. Compared with the control group, NO production of the training group also increased slightly but not significant before and after modified Bruce exercise treadmill test. This result might be caused by the small number of samples; or maybe short-term low-intensity exercise training was not adequate enough to stimulate the enhancement of NO significantly; meanwhile, it was adequate to control the production of ROS, along with the other internal antioxidants.

Several studies had showed that long-term regular exercise training with high intensity could increase production of NO in the endothelial blood vessel significantly.^{16–22} There are no studies that have been conducted until now about molecular effects of short-term low-intensity exercise training, such as ROS and NO production, especially in patients hospitalized with AMI. There are no reports about this kind of study and our study is the first reporting it.

Fig. 1 The effect of modified Bruce exercise treadmill test on F2-isoprostane and nitric oxide production in patients with acute myocardial infarction.

Fig. 2 Effects of modified Bruce exercise treadmill test on exercise duration and maximal capacity in training group of patients with acute myocardial infarction.

Fig. 3 Effects of modified Bruce exercise treadmill test on heart rate and systolic blood pressure in patients with acute myocardial infarction.

Goto et al³⁴ showed that long-term regular exercise training with high intensity for healthy persons could increase oxidative stress, whereas regular exercise training with moderate intensity was more favorable to increase NO activity. But Goto et al also showed that regular exercise training with low-intensity for healthy persons could not change F2-isoprostane and NO production significantly. It seemed that NO had an important role in controlling ROS production.

Our investigation showed that on the basis of multivariate analyses—before short-term low-intensity exercise training sessions—baseline NO was as a negative predictor variable for F2-isoprostane in patients hospitalized with AMI. The higher the NO concentration, the lower the F2-isoprostane. Clinically, short-term low-intensity exercise training caused neither any significant side effects nor complications for patients with AMI. In this study, maximal physical capacity of the training group on modified Bruce exercise treadmill test increased significantly. The maximal heart rate on such exercise treadmill test in the training group was lower than in the control group. The maximal systolic blood pressure was also lower in the training group.

Although short-term low-intensity exercise training had favorable effects on physical capacity, heart rate, and systolic blood pressure, the tendency of increasing NO level was not significant yet. As gaseous molecule, NO had an important role in controlling all of vasoconstriction and vasodilatation signals quickly, especially catecholamine and sympathetic nerve signals. However, NO might be adequate enough to control ROS production. NO was obviously a negative predictor variable for F2-isoprostane.

In conclusion, short-term low-intensity exercise training sessions for patients hospitalized with AMI appeared to be safe and clinically showed improvements. From molecular aspects, NO was a negative predictor variable for F2-isoprostane in patients hospitalized with AMI and seemed to have a significant role of compensation mechanisms in controlling ROS production.

Disclosures

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