Impact of Endovascular Therapy on Oxidative Stress in Patients With Peripheral Artery Disease

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Background: Atherosclerosis is believed to be caused by oxidative stress. Endovascular therapy (EVT) is effective for claudication of patients with peripheral artery disease (PAD). However, its effect on oxidative stress in PAD patients is unknown. Here, the impact of EVT on oxidative stress in PAD patients is investigated.

Methods and Results: Twenty-five PAD patients (Rutherford stage II or III) who underwent EVT were enrolled. The levels of diacron-reactive oxygen metabolite (d-ROM; an oxidative stress marker), ankle-brachial index (ABI), and maximum walking distance at baseline and at 3 months after EVT were measured. As compared with baseline values, the maximum walking distance and ABI improved significantly after EVT (109.9±104.2 vs. 313.7±271.8 m, P<0.0001; 0.61±0.15 vs. 0.91±0.13 m, P<0.0001, respectively). The improved exercise capacity and arterial flow induced a significant decrease in d-ROM levels (from 472.8±64.8 to 390.2±46.7 U.CARR, P<0.0001). The decrease in d-ROM levels after EVT was more prominent in PAD patients with a high baseline d-ROM level. The increased ABI (r=0.524, P=0.0007) and maximum walking distance (r=–0.416, P=0.039) after EVT were significantly correlated with the decreased d-ROM levels.

Conclusions: The improved exercise capacity and peripheral blood flow induced by EVT decreases oxidative stress in PAD patients. (Circ J 2014; 78: 1445–1450)

Key Words: Exercise; Oxidative stress; Peripheral artery disease; Revascularization

Atherosclerosis is a systemic disease that affects all major vascular territories. Peripheral artery disease (PAD) is an atherosclerotic disease affecting mainly the lower extremities that causes atherosclerosis, and its incidence continues to increase worldwide. This disorder affects 8–12 million individuals in the U.S. and is increasingly prevalent in Europe and Asia. Endovascular therapy (EVT) has been established as a primary therapy for patients with PAD. The purpose of EVT is to improve walking distance and leg symptoms, including intermittent claudication and resting leg pain. EVT and optimal medical treatment can result in the improvement of leg symptoms in most patients. However, PAD patients often have atherosclerosis-related complications, such as coronary artery disease and cerebrovascular disease, which affect the overall mortality. In the REACH registry, the rate of complications for patients with coronary artery disease and cerebrovascular disease was 38.5% and 9.8%, respectively, with a combined complication rate of 13.1%. Revascularization therapy, including EVT, is considered to be limited to the vascular bed; therefore, it is thought that EVT cannot prevent the development of other systemic vascular bed diseases.

Editorial p1327

The association between oxidative stress and the development of atherosclerosis has been investigated in several recent reports. Oxidative stress plays many roles in the pathophysiology of atherosclerosis, including dyslipidemia, which leads to atheroma formation, diabetes mellitus, endothelial dysfunction, plaque rupture, and myocardial ischemic injury. Furthermore, it is correlated with metabolic syndrome, which is a known risk factor for atherosclerosis. Moreover, metabolic syndrome is correlated with symptomatic and functional outcomes in PAD patients. Oxidative stress could serve as both an etiological factor as well as a predictor for atherosclerosis. However, the strategies for improving oxidative stress using antioxidant agents remain unclear. Performing appropriate exercise has been reported to be effective in improving oxidative stress. Semba et al described a correlation between oxidative stress and walking disability among older women.
However, PAD patients show a decreased walking capacity due to the presence of leg pain. Thus, impaired walking capacity in PAD patients might increase their oxidative stress levels.

With regard to patients with PAD who exhibit intermittent claudication, EVT has been shown to effectively increase walking capacity. Impaired walking ability has several important clinical implications. Diminished physical activity in daily life predicts higher overall mortality in patients with PAD. Functional measures including the 6-min walk test and treadmill walking time have been associated with increased mortality and risk of cardiovascular events in patients with PAD. Together, these findings suggest that EVT and augment exercise performance in patients with PAD might have a wide range of healthy benefits. However, it is not clear whether EVT additionally contributes to a reduction in the cardiovascular risk, which is a major therapeutic goal in PAD patients.

We hypothesize that improved exercise capacity, induced by EVT, decreases the oxidative stress level in PAD patients. Therefore, in the present study, we investigated the effects of EVT on oxidative stress and the relationship between the changes in oxidative stress levels and the maximum walking distance in PAD patients.

**Methods**

**Patient Population**

A total of 25 patients with PAD who underwent EVT in our institution were enrolled in this retrospective study. All the patients experienced intermittent claudication (Rutherford stage II and III). Patients were excluded if they had untreated coronary artery disease, symptomatic heart failure, or cancer. Patients who required hemodialysis were also excluded, because hemodialysis has been reported to affect oxidative stress levels. We evaluated the medication history before and after EVT; these were observed to be unchanged. The medication history was evaluated because certain medical agents can influence oxidative stress.

**Sampling and Measurement**

We determined the levels of diacron-reactive oxygen metabolite (d-ROM), a marker of oxidative stress, from the venous serum samples collected just before and at 3 months after EVT. The d-ROM level is known to be proportional to the serum hydroperoxide concentration (Figure 1). Hydroperoxides are the peroxidation products of proteins, peptides, amino acids, lipids, and fatty acids. In the presence of peroxides, the measurement of the d-ROM level is based on the ability of transition metals to catalyze the formation of free radicals, which are trapped by an alkylamine. The alkylamine reacts to form a colored radical that can be detected at 505 nm. The d-ROM levels were determined by the Free Radical Ejective Evaluator (Diacon International, Grosseto, Italy) using commercial assay kits (Diacon International srl, Grosseto, Italy). The results are expressed in conventional units called U.CARR (Carratelli units; 1 U.CARR corresponds to 0.8 mg/L H2O2). The d-ROM level in 25 healthy male control subjects (age 65.5 ± 5.6 years) from our institution is 367.7 ± 56.2 U.CARR.

**Statistical Analysis**

Continuous variables are presented as mean ± standard deviation, and categorical variables are expressed as a number and percentage.
percentage. Continuous variables were compared using the 2-sided paired t-test, and categorical variables were compared using the chi-square test or Fisher’s exact test, as appropriate. The relationship between the continuous variables was investigated using Pearson’s correlation coefficient test. Results with a P<0.05 and a r>0.4 were considered to be statistically significant. All analyses were performed using SPSS statistical software, version 13.0 (SPSS Inc, Chicago, Illinois, USA).

Results

The baseline characteristics of the patients are described in Table 1. The average age of the 25 patients was 73.6±7.1 years, and 23 patients (92%) were male. Hypertension was observed in 20 patients (80%), dyslipidemia was observed in 17 patients (68%), and diabetes mellitus (DM) was observed in 10 patients (40%). A history of smoking was recorded in 21 patients (84%). Moreover, chronic kidney disease was observed in 4 patients (16%); for all the patients, the average serum creatinine level was 0.93±0.40 mg/dl and the estimated glomerular filtrated rate was 63.16±20.58 ml·min⁻¹·1.73 cm⁻². With regard to intermittent claudication, 4 (16%) and 21 (86%) out of 25 patients were classified as Rutherford stage II and III, respectively. The average ABI and maximum walking distance values were 0.61±0.15 and 109.9±104.2 m, respectively.

Table 2 describes the characteristics of the target lesions. Of all the target lesions, 14 (56%) were present in the iliac artery (56%) and 11 (44%) were present in the superficial femoral artery. Moreover, based on the classification of the TransAtlantic Inter-Society Consensus II (TASC II), 7 lesions (28%) were Type A, 2 lesions (8%) were Type B, 5 lesions (20%) were Type C, and 11 lesions (44%) were Type D. The average lesion length and lesion width were 97.2±61.4 mm and 6.04±1.17 mm, respectively. Stenting 25 (100%) Table 3 shows a comparison of clinical parameters before

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<thead>
<tr>
<th>Table 3. Comparison of the Clinical Characteristics Before and 3 Months After EVT</th>
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<tbody>
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<td>Before EVT</td>
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<td>C-reactive protein (mg/dl)</td>
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<td>Serum creatinine (mg/dl)</td>
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<td>Hemoglobin (g/dl)</td>
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<td>Maximum walking distance (m)</td>
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<td>ABI</td>
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<td>d-ROM (U.CARR)</td>
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Values are presented as mean±standard deviation.

EVT, endovascular therapy. Other abbreviations as in Table 1.

![Figure 2](image-url)
Figure 3. Correlation between delta d-ROM and baseline d-ROM. The differences in the d-ROM levels before (at baseline) and at 3 months after EVT were correlated with the baseline d-ROM levels (P<0.0001, r=0.707). d-ROM, diacron-reactive oxygen metabolite; EVT, endovascular therapy.

Figure 4. Correlation between delta d-ROM and delta ABI. The differences in the d-ROM levels before (at baseline) and at 3 months after EVT were significantly correlated with the differences in ABI before (at baseline) and at 3 months after EVT (P=0.007, r=0.524). d-ROM, diacron-reactive oxygen metabolite; ABI, ankle-brachial index; EVT, endovascular therapy.

Figure 5. Correlation between delta d-ROM and delta walking distance. The differences in the d-ROM levels before (at baseline) and at 3 months after EVT were correlated with the differences in walking distance before (at baseline) and at 3 months after EVT (P=0.039, r=0.416). d-ROM, diacron-reactive oxygen metabolite; EVT, endovascular therapy.
and at 3 months after EVT. The serum C reactive protein (CRP), serum creatinine, and hemoglobin levels before EVT did not significantly differ from those at 3 months after EVT. However, the maximum walking distance and ABI values increased significantly at 3 months after EVT as compared to the values before EVT. At baseline, the d-ROM level in PAD patients (472.8±64.8 U.CARR) were higher than the d-ROM levels in healthy control subjects (367.7±56.2 U.CARR); however, the d-ROM levels decreased significantly at 3 months after EVT (472.8±64.8 vs. 390.2±46.7 U.CARR, P<0.0001; Figure 2).

A delta value was defined as the difference in the value before (at baseline) and after EVT. As shown in Figure 3, we observed a significant negative correlation between delta d-ROM levels and baseline d-ROM levels (P<0.0001, r=–0.707). The decrease in d-ROM levels was more prominent in patients with a higher baseline d-ROM level.

The correlation between delta d-ROM and delta ABI is shown in Figure 4. The increased ABI level at 3 months after EVT was significantly correlated with the decreased d-ROM level at 3 months after EVT (P=0.007, r=0.524). Figure 5 shows the correlation between delta d-ROM and the delta maximum walking distance. The improvement in the maximum walking distance at 3 months after EVT was significantly correlated with the decreased d-ROM levels (P=0.039, r=–0.416).

**Discussion**

In the present study, we showed that EVT successfully improved maximum walking distance and ABI in PAD patients. We also demonstrated that their oxidative status—as indicated by the d-ROM levels—was significantly improved after EVT and that the changes in oxidative stress levels were significantly associated with the improvement in the maximum walking distance and ABI.

With the evolution of the EVT technique, the number of patients undergoing EVT has increased, making it a commonly performed procedure in recent times. Increasing evidence suggests that the primary and long-term patency rate achieved with nitinol stents for iliac and superficial femoral arterial lesions is superior to that achieved by conventional balloon angioplasty alone. EVT is known to improve the symptoms of PAD patients, including intermittent claudication and resting leg pain. After undergoing EVT, the patients can reportedly walk longer distances, mainly due to improvement in intermittent claudication. In the present study, we were able to describe an additional beneficial effect of EVT on the oxidative status of these patients. To the best of our knowledge, this is the first study to show that EVT improves oxidative status in PAD patients.

Oxidative stress is caused by an imbalance between the generation of reactive oxygen species (ROS) and the capacity of the antioxidant defense system. Although there is a lack of appropriate epidemiological markers to measure oxidative stress, a few markers have been examined specifically with respect to PAD. In the present study, we used the d-ROM test, which facilitates the measurement of hydroperoxides, to detect the level of oxidative stress. The usefulness of the d-ROM test for evaluating the correlation between increased levels of systemic inflammation and oxidative stress has been reported in previous clinical studies. As compared to other conceptually similar tests for assessing oxidative status in plasma samples, d-ROM test values correlate directly and significantly with plasma 8-isoprostane levels in healthy subjects. A highly significant positive correlation has also been found between the d-ROM test and the FOX assay, a well-established test for plasma lipid hydroperoxides, in controls and hemodialysed patients. Therefore, in this study, we used the d-ROM test to investigate the effect of EVT on oxidative status in PAD patients.

The favorable effects of EVT on the oxidative status in PAD patients can be attributed to improvements in both arterial flow—reflected by the improvement in the ABI—and perfusion to peripheral tissues. In the present study, the improvement in the ABI was significantly correlated with the delta d-ROM levels. However, the favorable effects of EVT on the oxidative status might also be partly attributed to the improvement in the walking distance. Several studies have reported a correlation between regular physical activity and oxidative stress. In the present study, we also noted a significant correlation between baseline d-ROM and delta d-ROM levels. Thus, the decrease in d-ROM levels was more prominent in patients with a higher baseline d-ROM level.

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**References**


