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Original article

Effect of olmesartan on the levels of circulating endothelial progenitor cell after drug-eluting stent implantation in patients receiving statin therapy

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ABSTRACT

Background: The endothelial progenitor cell (EPC) plays an important role in repairing vascular injury. Statins and angiotensin II receptor blockers increase the level of circulating EPCs. However, it is unknown whether the angiotensin II receptor blocker olmesartan synergistically acts with statins to increase the levels of circulating EPCs. Moreover, the association between the levels of circulating EPCs and endothelial dysfunction after implantation of drug-eluting stents (DESs) has not been evaluated.
Methods: Nine patients with stable coronary artery disease underwent percutaneous coronary intervention (PCI) and received DES implantation. All patients received olmesartan in addition to statin therapy after PCI. The dose of olmesartan was based on the physician's discretion as per the patients' blood pressure. The levels of circulating EPCs were analyzed at baseline, post-PCI, and 1, 2, 3, and 8 months after PCI. Coronary angiography and the acetylcholine provocation test were performed on all patients at 8 months.
Results: Although the angiotensin II level significantly changed, the levels of circulating EPCs did not change during 8 months of olmesartan treatment (3.1 ± 0.6 cells/ml, 2.5 ± 0.8 cells/ml, 2.0 ± 0.6 cells/ml, 2.9 ± 0.9 cells/ml, 3.0 ± 0.4 cells/ml, 3.4 ± 0.8 cells/ml, $p = 0.64$). The patients were subsequently divided into two groups based on whether the level of circulating EPCs was less or greater than 4 cells/ml at 8 months. There were no significant differences in the mean vessel diameter of each segment (proximal, proximal edge, distal edge, and distal) after the acetylcholine provocation test between the two groups.
Conclusions: Low-to-moderate doses of olmesartan might not increase the level of circulating EPCs in patients receiving statin therapy. There might be no association between the levels of circulating EPCs and the degree of coronary vasospasm in the acetylcholine provocation test 8 months after DES implantation.

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Introduction

Circulating endothelial progenitor cells (EPCs) play an important role in repairing vascular injury through rapid endothelial regeneration [1,2]. EPCs are a key component of vascular healing after percutaneous coronary intervention (PCI) [3]. Nonetheless, cardiovascular risk factors are associated with reduced number of circulating EPCs [4–7]. Statins and certain antihypertensive drugs, such as angiotensin II receptor blockers (ARBs), increase the levels of circulating EPCs [4,8–11]. Atorvastatin increases the level of circulating EPCs by a factor of 3 in patients with stable coronary artery disease [8]. Olmesartan also increases the level by a factor of 2 in patients with type II diabetes [11]. However, to the best of our knowledge, no data are available on whether these drugs act synergistically. Therefore, we evaluated the effect of olmesartan on the level of circulating EPCs after PCI in patients receiving statin therapy. Because endothelial dysfunction can occur following the implantation of drug-eluting stents (DESs), we investigated whether this adverse event is associated with the number of circulating EPCs.

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Methods

Patients and study protocol

Nine patients with stable coronary artery disease underwent PCI, and DES was implanted in the lesion. Angiotensin-converting enzyme inhibitors and ARBs were not prescribed before PCI; however, statins were prescribed at least 3 months before the procedure for all patients [atorvastatin 10 mg/day (n = 3), rosuvastatin 2.5 mg/day (n = 2), rosuvastatin 5 mg/day, rosuvastatin 10 mg/day, pitavastatin 1 mg/day, and pitavastatin 2 mg/day (n = 1)]. Olmesartan was started after PCI, and the dose was based on the physician's discretion according to the patient's blood pressure. We determined the number of circulating EPCs (baseline, post-PCI, and after 1, 2, 3, and 8 months) and the levels of angiotensin II (baseline, 1, 3, and 8 months), inteleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), and P-selectin (baseline, post PCI, and after 1, 3, and 8 months). Follow-up coronary angiography and the acetylcholine provocation test were performed at 8 months for all patients. The primary endpoint of the present study was the level of circulating EPCs during 8 months. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or use of an antihypertensive drug. Dyslipidemia was defined as low-density lipoprotein cholesterol >100 mg/dl, high-density lipoprotein cholesterol ≤50 mg/dl, triglycerides ≥150 mg/dl, or medication use. This study was reviewed and approved by the local Ethics Review Committee, and written informed consent was obtained from all patients.

Interventional protocol and quantitative coronary analysis

All interventions were performed using standard techniques. The type of DES was chosen according to the physician's discretion. All patients were advised to continue treatment with dual antiplatelet therapy (aspirin 100 mg/day and clopidogrel 75 mg/day) for 12 months after PCI and lifelong daily use of aspirin. The acetylcholine provocation test was performed along with follow-up angiography after 8 months. After baseline angiography, incremental doses of acetylcholine (50 and 100 µg/20 s into the left coronary artery and 25 and 50 µg/20 s into the right coronary artery) were infused directly through the catheter. After an

additional 5 min, intracoronary isosorbide dinitrate (5 mg/10 s) was infused into the right and left coronary arteries. Qualitative and quantitative coronary angiography was evaluated at an independent angiographic core laboratory (Cardiocore, Tokyo, Japan) using a Coronary Angiography Analyses System (Medis QAngio XA 7.1, Leiden, The Netherlands). Baseline, post-procedure, and follow-up angiograms were obtained from all patients. The target segment was defined as the entire segment involving the implanted stent and the 5-mm proximal and distal edges adjacent to the stent. In the acetylcholine provocation test, a reference vessel not related to the stent lesion was analyzed (5–15 mm proximal and distal to the stent edges). We divided the enrolled patients into two groups based on the level of circulating EPCs (4 cells/ml) and compared the results of the acetylcholine provocation test between the two groups.

Level of circulating EPCs

A 20-ml sample of peripheral blood was obtained from each patient, and the number of circulating EPCs was determined within 24 h (SRL, Tokyo, Japan) using a fluorescence-activated cell sorter (FACScan, Becton Dickinson, Franklin Lakes, NJ, USA). EPCs were identified by the presence of CD34, CD45, CD133, and CD133 antigens (Fig. 1).

Statistics

Continuous variables are expressed as mean ± standard deviation (SD) and compared using Student's t test. The values for all blood samples for all time points were compared using repeated measure analysis of variance (ANOVA). All statistical tests were two-tailed. Statistical significance was defined as p = 0.05.

Results

Baseline characteristics and outcomes

Nine patients were enrolled in this study. Table 1 presents their baseline characteristics. Their mean age was 70 years. All patients had dyslipidemia and received statin therapy at least 3 months before the procedure. Patients received 40 mg (n = 3) or 20 mg doses (n = 6) of olmesartan during follow-up. Lesion and procedural characteristics are presented in Table 2. Sirolimus-eluting and

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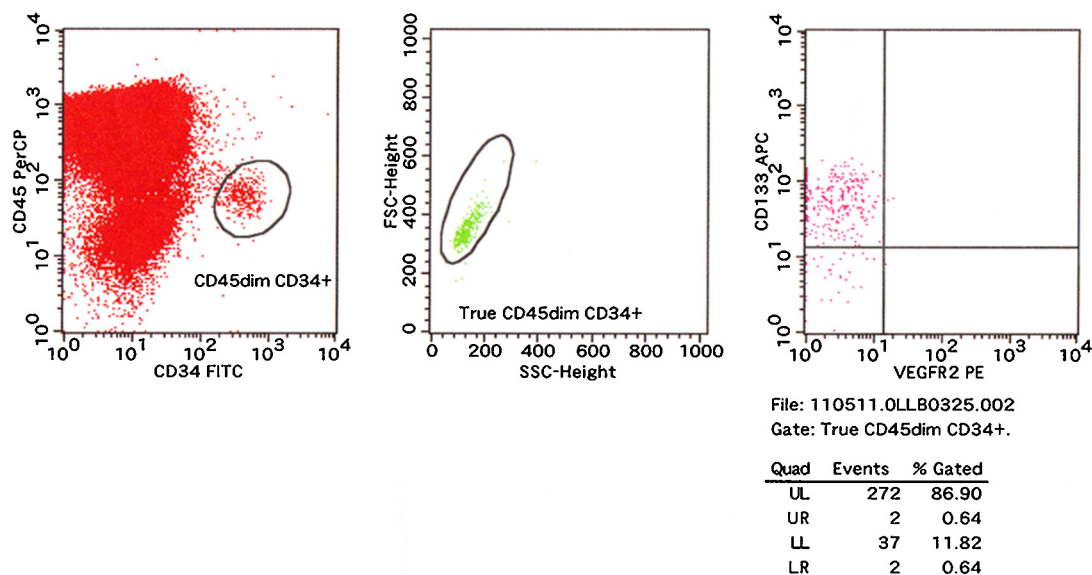| Quad	Events	% Gated
UL	272	86.90
UR	2	0.64
LL	37	11.82
LR	2	0.64

Fig. 1. The number of endothelial progenitor cells was determined using flow cytometry to detect CD34, CD45, CD133, and VEGFR2 antigens.

Table 1
Patient baseline characteristics.

	$N = 9$
Age	70.0 ± 10.4
Male	6 (66.7%)
Hypertension	6 (66.7%)
Dyslipidemia	9 (100%)
Family history of CAD	2 (22.2%)
Diabetes mellitus	2 (22.2%)
Smoking	4 (44.4%)
Multi-vessel disease	4 (44.4%)
Creatine (mg/dl)	0.78 ± 0.15
eGFR (ml/min)	68.9 ± 7.9
LDL (mg/dl)	100.6 ± 32.3
High sensitivity CRP (mg/l)	0.63 ± 1.32

CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; LDL, low density lipoprotein; CRP, C-reactive protein.

everolimus-eluting stents were implanted in four and five patients, respectively.

Number of circulating EPCs and levels of inflammation markers

During 8 months, the angiotensin II levels changed significantly (6.6 ± 1.1 pg/ml, 14.0 ± 3.4 pg/ml, 24.6 ± 9.0 pg/ml, 9.2 ± 1.1 pg/ml, $p = 0.02$, Fig. 2) but the level of circulating EPCs did not change (3.1 ± 0.6 cells/ml, 2.5 ± 0.8 cells/ml, 2.0 ± 0.6 cells/ml, 2.9 ± 0.9 cells/ml, 3.0 ± 0.4 cells/ml, 3.4 ± 0.8 cells/ml, $p = 0.64$; Fig. 3A). The number of circulating EPCs increased in four patients and decreased in five patients at 8 months compared with baseline (Fig. 3B). The levels of MCP-1 significantly changed during 8 months because of the low level after the procedure (Fig. 4). In contrast, the levels of IL-6, TNF-α, and P-selectin did not significantly change during follow-up (Fig. 4).

Quantitative coronary angiography and acetylcholine provocation test

All patients underwent coronary angiography at 8 months, and the mean in-stent and in-segment late luminal losses were 0.05 ± 0.40 mm and 0.03 ± 0.42 mm, respectively. Binary restenosis was not detected in any patient (Table 3). When the patients were divided into two groups based on the level of circulating EPCs at 8 months (EPC level greater or less than 4 cells/ml), the change

Table 2
Lesion and procedural characteristics.

	$N = 9$
Treated vessel	
RCA	1 (11.1%)
LAD	3 (33.3%)
LCX	5 (55.5%)
Lesion type	
A	0 (0%)
B1	1 (11.1%)
B2	6 (66.6%)
C	2 (22.2%)
Bifurcation lesion	6 (66.6%)
Diffuse lesion	2 (22.2%)
Pre	
Lesion length (mm)	18.04 ± 7.69
Reference vessel diameter (mm)	2.60 ± 0.66
Minimal lumen diameter (mm)	0.82 ± 0.26
Diameter stenosis (%)	67 ± 12
DES type	
Sirolimus	4 (44.4%)
Everolimus	5 (55.5%)
Average stent diameter (mm)	3.01 ± 0.32
Average stented length (mm)	25.2 ± 7.9
Stent/lesion	1.2 ± 0.4

RCA, right coronary artery; LCX, left circumflex artery; LAD, left anterior descending artery.
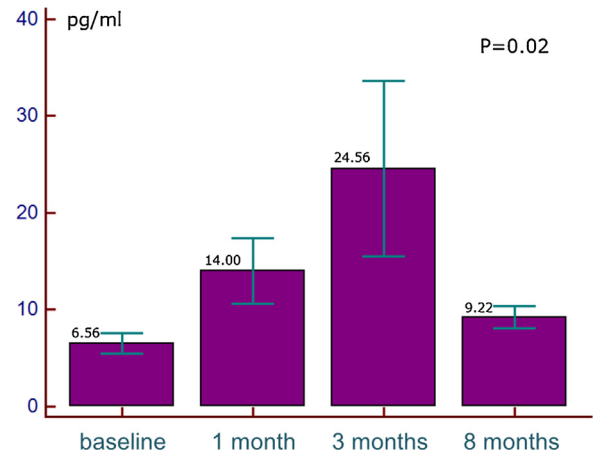

Fig. 2. Changes in angiotensin-II levels. The angiotensin II level increased during the first 3 months of treatment and decreased at 8 months.

(%) in the mean vessel diameter after intracoronary acetylcholine and isosorbide dinitrate injections did not significantly change for each segment (proximal, proximal edge, distal edge, and distal segments) between the two groups (Fig. 5).

Discussion

The principal findings of the present study are that low-to-moderate doses of olmesartan did not have a synergistic effect with statins to increase the level of circulating EPCs in patients receiving statin therapy, and there was no association between the number of circulating EPCs and the degree of coronary vasospasm detected using the acetylcholine provocation test after DES implantation.

ARBs lower blood pressure and induce pleiotropic effects, including anti-inflammation [12]. One mechanism that accounts for these pleiotropic effects may be increasing the level of circulating EPCs. Angiotensin II accelerates the onset of EPC senescence by increasing GP91-PHOX levels through activation of the angiotensin II receptor [13]. Further, angiotensin II promotes the vascular endothelial growth factor-induced proliferation and network formation of human EPCs [14]. ARBs have the potential to delay EPC senescence and promote EPC proliferation. Olmesartan significantly reduced the expression of vascular-inflammation markers (high sensitivity C-reactive protein, IL-6, MCP-1, and TNF-α) in patients with essential hypertension and significantly increased the number of circulating EPCs in patients with type II diabetes [11,12]. However, we did not detect these effects in this study.

These results may be explained as follows. First, we enrolled patients receiving statin therapy. Statins reduce inflammation and increase the number of circulating EPCs [4], and the mechanisms responsible for these effects may be shared by statins and ARBs. Therefore, the effect of olmesartan is eliminated in patients receiving statin therapy. Second, in a previous study, an increased EPC level was observed in patients with type II diabetes patients but not in healthy subjects [11]. The prevalence of diabetes was only 20% in our study. Third, our study used either 20 mg or 40 mg olmesartan compared with 80 mg in the previous study [11]. Although the angiotensin II level significantly changed, this dose may have been insufficient to increase the number of circulating EPCs. High doses of olmesartan may be required to increase the level of circulating EPCs, particularly in patients who have already received statin therapy. Fourth, ARBs theoretically improve the level of circulating EPCs [12–14]. Other ARBs might increase the level of circulating EPCs even in patients on statin therapy.

Patients often experience vascular dysfunction following DES implantation [15,16]. Angina sometimes occurs in these patients
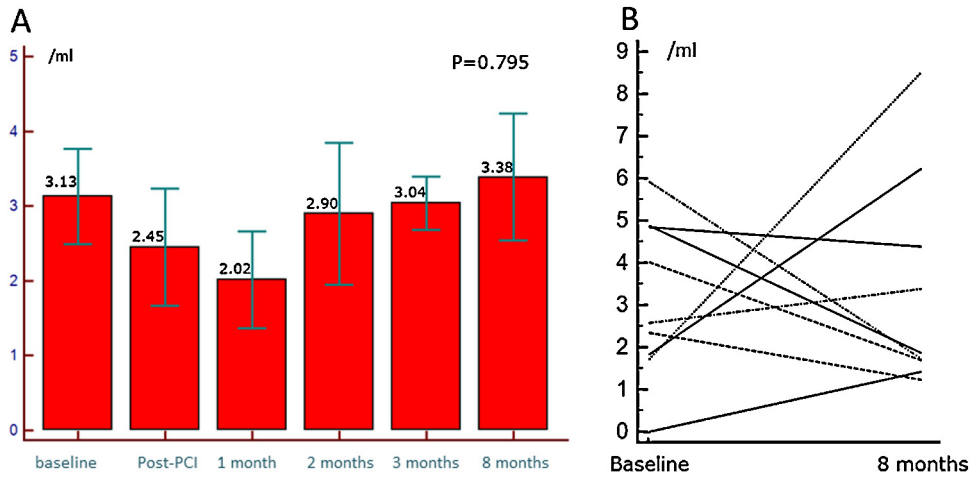
Fig. 3. (A) Change in the level of circulating endothelial progenitor cells (EPCs). The level of circulating EPCs did not significantly change. (B) The level of circulating EPCs at baseline and 8 months in each patient. The level of circulating EPCs increased in four patients and decreased in four patients. PCI, percutaneous coronary intervention.

without in-stent restenosis, and severe coronary spasms can be life-threatening [17,18]. These adverse effects of a DES implant are related to endothelial dysfunction [19]. However, to the best of our knowledge, there is no report investigating the predictors of endothelial dysfunction after DES implantation.

Circulating EPCs mediate ongoing endothelial repair. The level of circulating EPCs may serve as a surrogate biologic marker for vascular endothelial function [6]. We hypothesized that the number of circulating EPCs determines whether vascular dysfunction

occurs after DES implantation. However, in this study of a small number of patients, the level of circulating EPCs was not associated with vascular dysfunction indicated using the acetylcholine provocation test. Considering the average level of circulating EPCs in the present study (3.4 cells/ml), we defined the cut-off point as 4 cells/ml. A larger number of circulating EPCs may be required to prevent vascular dysfunction after DES implantation. Another possibility is that there is no association between the level of circulating EPCs and vasospastic response. To the best of our
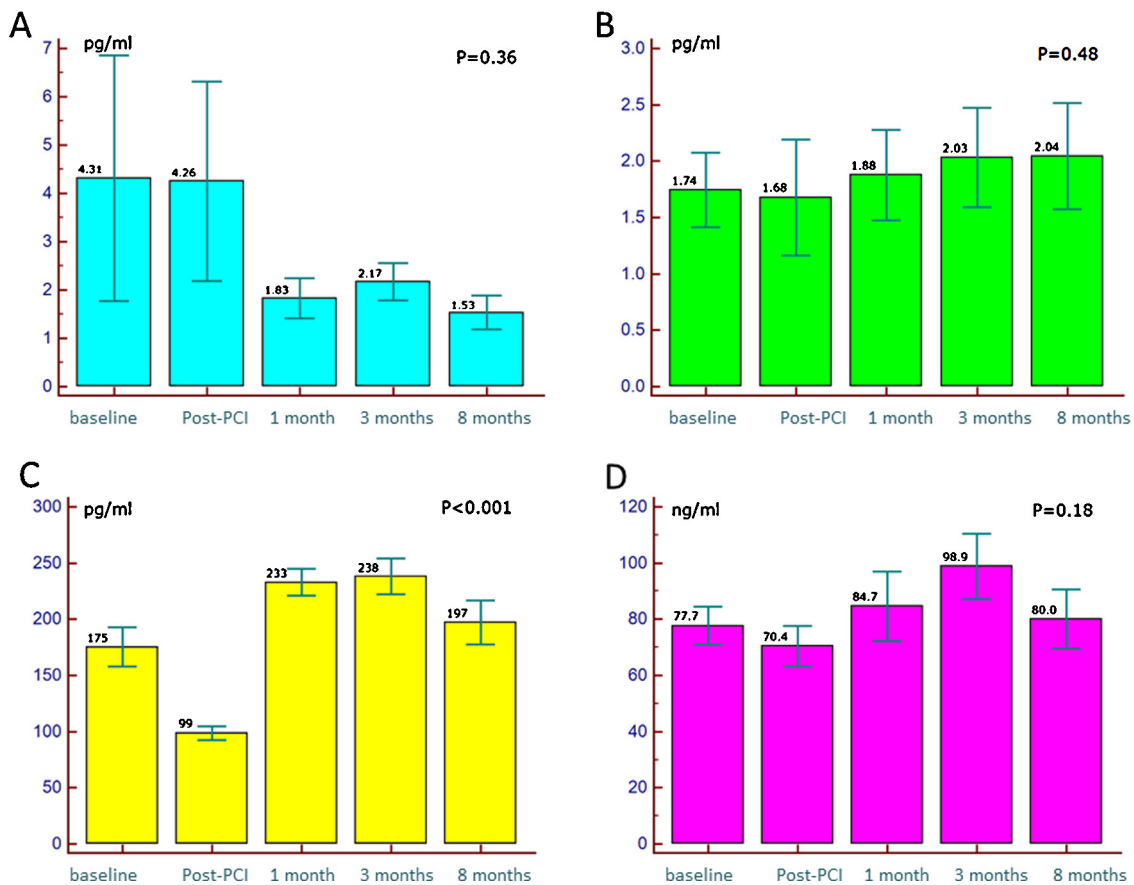

Fig. 4. Changes in interleukin-6 (A), tumor necrosis factor-α (B), monocyte chemotactic protein-1 (MCP-1) (C), and P-selectin (D) levels. A significant change was observed only for MCP-1. PCI, percutaneous coronary intervention.

Table 3
Quantitative coronary angiography results.

	In-lesion	Proximal edge	In-stent	Distal edge
Reference diameter				
Post-procedure (mm)	2.99 ± 0.60	3.28 ± 0.51	3.13 ± 0.52	2.76 ± 0.57
Follow-up (mm)	3.06 ± 0.70	3.37 ± 0.46	3.09 ± 0.65	2.79 ± 0.63
Minimal lumen diameter				
Post-procedure (mm)	2.26 ± 0.67	2.79 ± 0.57	2.49 ± 0.60	2.29 ± 0.69
Follow-up (mm)	2.23 ± 0.62	2.77 ± 0.63	2.43 ± 0.59	2.23 ± 0.66
Percent diameter stenosis				
Post-procedure (%)	25 ± 10	15 ± 8	21 ± 10	18 ± 13
Follow-up (%)	28 ± 7	19 ± 9	21 ± 10	20 ± 13
Late loss (mm)	0.03 ± 0.42	0.02 ± 0.18	0.05 ± 0.40	0.06 ± 0.15
Binary restenosis rate (%)	0	0	0	0
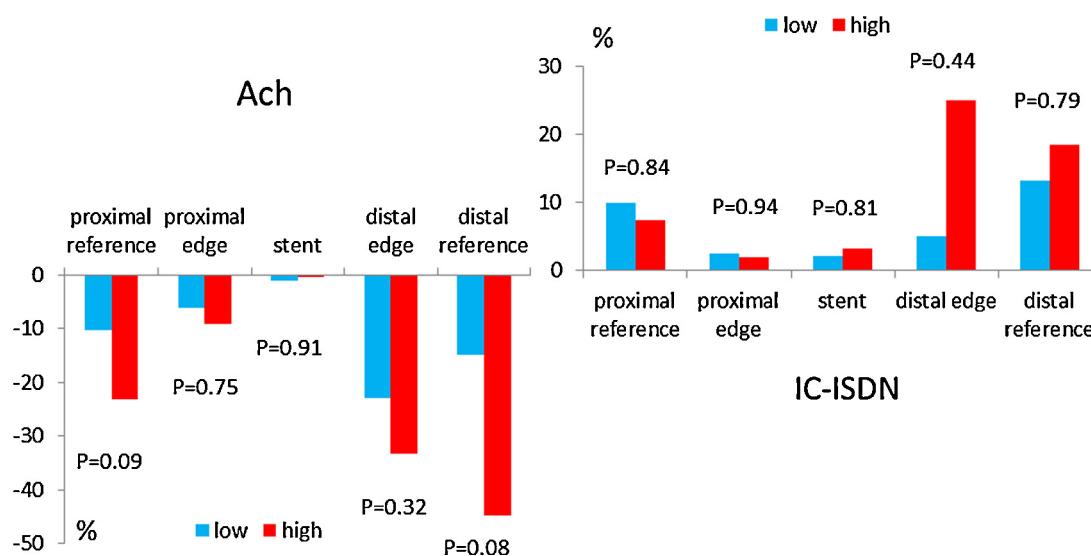
Fig. 5. Percentage change in mean vessel diameter after intracoronary acetylcholine (Ach) and isosorbide dinitrate (IC-ISDN) injections. The nine patients were divided into two groups according to the level of circulating endothelial progenitor cells at 8 months (three patients in the high group, and six patients in the low group). There were no significant differences between the two groups.

knowledge, no study showed the association between the level of circulating EPCs and vasospastic response following DES implantation to date. A further larger study is warranted to evaluate this possibility.

There are several limitations to the present study. First, the sample size is small and statistically insufficient for comparing the two groups. As the protocol, we planned to enroll 15 patients in this study from April 2009 to March 2010. Although we extended the enrollment until March 2011, nine patients were finally enrolled. Thus the results should be considered exploratory and hypothesis generating. Second, the analysis of the level of circulating EPCs is currently not unified [4]. Therefore, the number of circulating EPCs cannot be generalized, and it is not possible to compare their numbers determined in this study with those reported in other studies. For example, flow cytometry and/or colony-forming assays have been used. Further, several cell-surface markers are used to identify EPCs, including CD45, CD14, CD117, CD133, CD34, CD31, CD105, Tie-2, and KDR. Finally, there were differences in the types of stent and the types and doses of statins administered to our patients, which may have differentially affected the level of circulating EPCs in each patient. However, to the best of our knowledge, the present study is the first to consecutively (six times) analyze the number of circulating EPCs before and after PCI and to evaluate the association between the level of circulating EPCs and vascular response in the acetylcholine test.

Conclusions

Moderate-to-low doses of olmesartan did not synergize with statins to increase the level of circulating EPCs in patients receiving statin therapy. There was no association between the level of circulating EPCs and the degree of coronary vasospasm in the acetylcholine provocation test 8 months after DES implantation. A further larger study is warranted to evaluate the association between the number of circulating EPCs and vascular dysfunction following DES.

Acknowledgment

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jjcc.2014.02.029.

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