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Mobilization of progenitor cells and assessment of vessel healing after second generation drug-eluting stenting by optical coherence tomography

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ABSTRACT

Background: Bone marrow-derived progenitor cells likely contribute to both endothelial- and smooth muscle cell-dependent healing responses in stent-injured vessel sites. This study aimed to assess mobilization of progenitor cells and vessel healing after zotarolimus-eluting (ZES) and everolimus-eluting (EES) stents.
Methods and results: In 63 patients undergoing coronary stent implantation, we measured circulating CD34 + CD133 + CD45low cells and serum levels of biomarkers relevant to stem cell mobilization. In 31 patients of them, we assessed vessel healing within the stented segment using optical coherence tomography (OCT) imaging. The CD34 + CD133 + CD45low cells increased 68 ± 59% 7 days after bare metal stent (BMS), 10 ± 53% after ZES ($P < 0.01$ vs BMS), 3 ± 49% after EES ($P < 0.001$ vs BMS), compared with baseline. Percent change in CD34 + CD133 + CD45low cells was positively correlated with that in stromal cell-derived factor (SDF)-1α ($R = 0.29$, $P = 0.034$). Percentage of uncovered struts was higher in the EES group (14.4 ± 17.3%), compared with the BMS (0.7 ± 1.3, $P < 0.01$) and ZES (0.4 ± 0.5, $P < 0.01$) groups. The change in CD34 + CD133 + CD45low cells showed positive correlation with OCT-quantified mean neointimal area ($R = 0.48$, $P < 0.01$). Finally, circulating mononuclear cells obtained from 5 healthy volunteers were isolated to determine the effect of sirolimus, zotarolimus and everolimus on vascular cell differentiation. The differentiation of mononuclear cells into endothelial-like cells was dose-dependently suppressed by sirolimus, zotarolimus, and everolimus.
Conclusions: Mobilization of progenitor cells was suppressed, and differentiation of mononuclear cells into endothelial-like cells was inhibited, in association with increased number of uncovered stent struts, even after second generation drug-eluting stenting. These data suggest that new approaches are necessary to enhance stent healing.

1. Introduction

Generational advances in drug-eluting stent (DES) technology have resulted in reduced rates of target lesion revascularization across broad patient and lesion subsets with improved safety with respect to stent thrombosis. However, concerns over incomplete stent healing even with second-generation DES persist because the annual rate of target lesion failure still remains at 2–4% annually, which is similar to the rates observed after implantation of bare metal stent (BMS) or first-generation DES [1]. From a vascular biology perspective, there is consensus that late lumen loss and neointimal thickening (i.e., restenosis) are the biological response to vascular injury characterized by a sequence of endothelial denudation, platelet deposition and inflammatory cell recruitment, smooth muscle cell migration and proliferation, and extracellular matrix deposition. Complete stent coverage and re-endothelialization are commonly viewed as markers of favorable vascular healing [2,3].

In the course of investigating vascular healing after stent deployment, multiple research laboratories, including our own, discovered

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that progenitor cells from bone marrow and other tissues serve as a source of both smooth muscle cell and endothelial cell precursors in the healing response [4]. These same progenitor cells play an essential role in angiogenesis [5,6]. We reported previously that CD34-positive (CD34+) cells, which include smooth muscle progenitor cells (SMPCs) as well as endothelial progenitor cells (EPCs), are mobilized into the circulation after stenting and are positively correlated with an increased risk of restenosis [7]. Specifically, circulating CD34+ cells increased 7–14 days after BMS deployment and was associated with late lumen loss and restenosis; first-generation sirolimus-eluting stent (SES) suppressed late lumen loss and CD34+ cell mobilization, raising the question of whether neointimal suppression is inexorably linked with impaired re-endothelialization. In the present study, we assessed mobilization of progenitor cells and vessel healing after second generation zotarolimus-eluting (ZES) and everolimus-eluting (EES) stents. In addition, we also investigated in-vitro pharmacological action of the drugs coated on the surface of DESs on the differentiation of progenitor cells into vascular cells.

2. Methods

2.1. Study design

The subjects included 63 patients (46 men and 17 women, aged 69 ± 9 yr) with stable coronary artery disease who underwent elective coronary stent implantation for organic lesions of single coronary artery, using any one of BMS, ZES or EES. Stent selection was based on operators' decision. Consequently, BMS was implanted in 20 patients, ZES in 19 and EES in the remaining 24 patients. All of the patients were receiving dual anti-platelet therapy with 81 mg of aspirin and 75 mg clopidogrel at least until the follow-up coronary angiography was performed. The follow-up coronary angiography was recommended for all patients at 12 months after stent implantation, and was performed earlier if necessary based on clinical indications. In all patients, peripheral blood sample was collected at baseline before stenting and on the day 7 post-stenting. The blood samples were immediately collected into tubes containing ethylene diaminetetraacetate (EDTA) and plain tubes. We measured the number of circulating progenitor cells including EPCs at baseline and on the day 7 post-stenting, using the EDTA blood. We also measured serum level of biomarkers relevant to stem cell mobilization using the plain tube blood. At the time of follow-up coronary angiography, we assessed re-endothelialization and neointimal growth at the site of stent placement using optical coherence tomography (OCT) imaging, in addition to quantitative coronary angiographic analysis (QCA). The local institutional review board in Dokkyo Medical University (Mibu, Tochigi, Japan) approved the study protocol, and written informed consent was obtained from each patient.

2.2. Measurement of circulating CD34+CD133+CD45low cells

We measured circulating CD34+CD133+CD45low cells, which include EPCs, using flow cytometry based on a previously described method [7,8,9] with minor modifications. In brief, the reagent mixture consisted of a nucleic acid dye (SY-III-8; Molecular Probe), a peridinine chlorophil protein (PerCP)-conjugated anti-CD45 (Becton Dickinson), a fluorescein isothiocyanate (FITC)-conjugated anti-CD34 (Becton Dickinson) and a phycoerythrin (PE)-conjugated anti-CD133 (Miltennyi Biotec). Isotype controls were used as negative controls based on the species and immunoglobulin (Ig) G control antibodies (IgG1 isotype control; Becton Dickinson). Flow cytometric analysis was performed using the FACS Calibur laser flow cytometer (Becton Dickinson) according to the manufacturer's instructions (Supplemental file 1). The absolute number of CD34+CD133+CD45low cells per milliliter was calculated based on the cells-to-the white blood cell count. To minimize any methodological variations, each sample was analyzed with two independent experiments, and the mean value was calculated.

2.3. Serum biomarker assays

In this study, we also measured serum levels of angiogenic biomarkers relevant to stem cell mobilization, such as stromal cell-derived factor (SDF)-1α, interleukin (IL)-8, and matrix metalloproteinase (MMP)-9, using enzyme-linked immunosorbent assay (ELISA). We used each commercially available ELISA kit, the Quantikine ELISA kit (R&D Systems) for SDF-1α (Human CXCL12/SDF-1α Immunoassay), IL-8 (Human CXCL8/IL-8 Immunoassay), and MMP-9 (Human MMP-9 Immunoassay). The procedure was performed according to the manufacturer's instructions. Each sample was assayed in duplicate standards and controls; high, medium, and low were included in each run. All results were reported within the linearity of the assay. The colorimetric reactions were read as the value of the optical density directly on the automatic microplate reader set to 450 nm.

2.4. Quantitative coronary angiography analysis

Coronary lesions were assessed by QCA using a computer-based CASS system (Pie Medical Instruments) before and immediately after stent implantation and at the time of follow-up coronary angiography. Lesion length, reference diameter and minimal lumen diameter were measured and late lumen loss (minimal lumen diameter after stenting minus minimal lumen diameter at follow-up angiography) was calculated.

2.5. Optical coherence tomography imaging and analysis

At the time of follow-up coronary angiography, OCT examination was performed using a frequency-domain system (C7-XR FD-OCT Intravascular Imaging System; LightLab Imaging). The cross-sectional neointimal area and the neointimal volume was calculated. In every cross-sectional image, neointimal coverage was assessed for all of the struts, and the percentage of uncovered struts to total struts in all OCT cross-sections was calculated [10]. The OCT analysis was performed by an independent investigator blinded to the study protocol in University Hospitals Harrington Heart & Vascular Institute, Case Western Reserve University School of Medicine (Cleveland, OH).

2.6. In-vitro experiment

We investigated in-vitro pharmacological action of the drugs coated on the surface of SES, ZES and EES, i. e., sirolimus, zotarolimus and everolimus, respectively, regarding differentiation of bone marrow-derived progenitor cells into vascular endothelial cells as well as vascular smooth muscle cells, using a previously described method [11] with minor modifications. Briefly, peripheral mononuclear cells were isolated from the peripheral blood of 5 healthy human volunteers (3 men and 2 women, aged 37 ± 8 yr). Mononuclear cells were cultured for 14 days in EGM-2 (Lonza) supplemented with hydrocortisone, bovine brain extract, vascular endothelial growth factor (VEGF) and fetal bovine serum (EPC medium) and also in HuMedia-SG2 (Kurabo) supplemented with platelet derived growth factor-BB and basic fibroblast growth factor (SMPC medium). Sirolimus (Sigma), zotarolimus (Toronto Research Chemicals) and everolimus (Sigma) were added to each culture medium at a concentration of 0.01, 0.1 and 1 nM each.

Immunocytochemistry was used to assay cultured cells for the expression of CD31 and von Willebrand factor (vWF) as endothelial cell markers, and for the expression of α-smooth muscle actin (α-SMA) and smooth muscle myosin heavy chain isoforms, SM1 and SM2, as smooth muscle cell markers, to identify endothelial- as well as smooth muscle-like cells. Primary monoclonal antibodies against human CD31 (JC70A; Dako Cytomation), vWF (F8/86; Dako Cytomation), α-SMA (1A4; Dako Cytomation), SM1 (3F8; Yamasa) and SM2 (1G12; Yamasa) were applied. Non-immune mouse IgG2a and IgG1 (Dako Cytomation) were used as negative controls. To visualize the immunoreactive