The Effect of Stem Cell Mobilization by Granulocyte-Colony Stimulating Factor on Neointimal Hyperplasia and Endothelial Healing After Vascular Injury With Bare-Metal Versus Paclitaxel-Eluting Stents

Hyun-Jai Cho, MD,*†‡ Tae-Youn Kim, BA,‡ Hyun-Ju Cho, MS,‡ Kyung-Woo Park, MD,*†‡ Shu-Ying Zhang, MD,‡ Ji-Hyun Kim, MS,‡ Sung-Hwan Kim, MD,‡‡ Joo-Yong Hahn, MD,*†‡ Hyun-Jae Kang, MD,*†‡ Young-Bae Park, MD,*†‡ Hyo-Soo Kim, MD, PhD*†‡ Seoul, Korea

OBJECTIVES
The goal of this study was to investigate the effect of mobilized stem cells by granulocyte-colony stimulating factor (G-CSF) on neointimal growth, the biologic impact on vascular healing process, and the utility of paclitaxel-eluting stent (PES) in this circumstance.

BACKGROUND
Questions have been raised on the safety of stem cell mobilization because of the tendency of neointimal overgrowth in a recent clinical trial, despite improvement of cardiac function.

METHODS
Rabbits underwent iliac artery injury with bare-metal stent (BMS) or PES and then received rhG-CSF or placebo for 6 days. Morphometric analysis and scanning electron microscopy for re-endothelialization were performed. The characteristics of mobilized peripheral blood mononuclear cells were determined in vitro, and the fate of these cells was evaluated by re-infusion with tagging in vivo.

RESULTS
At day 60 after stenting, neointimal overgrowth was observed at BMS with G-CSF. The tendency of neointimal overgrowth was substantially reduced on PES. Intriguingly, the delayed endothelial recovery on PES was restored to normal after G-CSF treatment. The G-CSF increased not only the endothelial progenitor cells, but also putative smooth muscle progenitor cells. Paclitaxel, at working concentration, preferentially inhibited proliferation of smooth muscle lineage cells rather than endothelial lineage cells.

CONCLUSIONS
Our findings demonstrate that G-CSF mobilizes putative vascular progenitor cells in peripheral blood, which induces neointimal overgrowth at stented vasculature. Unique differential action of paclitaxel results in the enhanced endothelial healing with reduced neointimal growth after G-CSF treatment, suggesting that drug-eluting stents might be the optimal modality for revascularization in cytokine-based stem cell therapy. (J Am Coll Cardiol 2006;48:366–74) © 2006 by the American College of Cardiology Foundation

The strategy of mobilizing stem cells from the bone marrow was initially contrived by hematologists to accelerate recovery of the bone marrow after cancer chemotherapy and to harvest hematopoietic stem cells from the peripheral blood for bone marrow transplantation (1,2).

In a recent experimental study, stem cells mobilized from the bone marrow were shown to improve cardiac function after myocardial infarction (3). In patients with coronary artery disease, it was shown that, despite reduced basal number and function of endothelial progenitor cells (EPCs) compared with healthy control subjects, granulocyte-colony stimulating factor (G-CSF) administration mobilized progenitor cells to the circulation and augmented EPC colony-formation (4). In a first-in-man trial (5), we reported that intracoronary infusion of peripheral blood stem cells mobilized by G-CSF after coronary stenting of culprit lesions with a bare-metal stent (BMS) improved systolic function and myocardial perfusion without significant systemic inflammation in patients with myocardial infarction. Unexpectedly, we observed the tendency of G-CSF to potentially promote neointimal growth at BMS. Our report and concern from other investigators (6) have raised questions on the safety of cytokine-induced mobilization. However, owing to the small number of patients enrolled in our study, it was difficult to come to a conclusion on the effects of G-CSF on neointimal formation and restenosis at BMS.
Furthermore, there have not been studies to evaluate the effect of G-CSF on neointimal growth or to elucidate possible mechanisms.

The principal cause of in-stent restenosis is neointimal hyperplasia caused by excessive proliferation and accumulation of vascular smooth muscle cells (SMCs) that arise from either the injured resident vascular tissue (7) or the bone marrow (8,9). In the clinical field, drug-eluting stents have shown efficacy in reducing neointimal hyperplasia through inhibition of SMCs and have mostly replaced BMS in practice (10). However, little is known about the biologic effect of mobilized stem cells on the vascular healing process after paclitaxel-eluting stent (PES) implantation.

Therefore, in the present study, we investigated whether G-CSF–induced stem cell mobilization from the bone marrow might have the potential to aggravate BMS neointimal hyperplasia and whether this can be reduced by PES in an animal model. Next, to elucidate its mechanism, we studied the characteristics of the mobilized cells and investigated whether these cells incorporate into the injured vessels leading to neointimal overgrowth. We also studied the mechanism for the enhanced endothelial healing with reduced neointimal overgrowth at PES after stem cell mobilization by G-CSF.

**METHODS**

**Animal care and vascular injury.** All animal experiments were performed after receiving approval from the Institutional Animal Care and Use Committee (IACUC) of the Clinical Research Institute in Seoul National University Hospital and complied with the National Research Council’s Guidelines for the Care and Use of Laboratory Animals (revised 1996).

Schematic diagram of study design and end points are illustrated in Figure 1.

Male New Zealand White rabbits (3 to 3.5 kg; Yonam Laboratory Animals, Cheonan, Korea) received a 1% cholesterol diet from at least 2 weeks prior to vascular injury. Total cholesterol levels were measured before injury. For peripheral blood analysis and cell culture, eight rabbits received either G-CSF (daily 70 μg subcutaneously, Dong-A Pharmaceutical, Korea) or placebo (human albumin) for 6 days.

To determine whether the cells mobilized by G-CSF actually incorporate into the injured vessels, mononuclear cells were isolated from a rabbit that received either G-CSF or placebo, tagged with DiI (Molecular Probes, Eugene, Oregon), and infused via arterial sheath into a recipient rabbit that received a balloon injury as previously described (11). Balloon injury was performed on bilateral iliac arteries with a 3.0 mm × 20 mm balloon. At 14 days after balloon injury with systemic cell infusion, rabbits were killed and their iliac arteries were harvested.

To evaluate re-endothelialization and neointimal hyperplasia after stenting, we deployed 20 BMS and 20 PES in the right and left iliac arteries, respectively, in 20 rabbits (n = 20, each stent) (BMS = EXPRESS, 2.75 mm × 16

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**Abbreviations and Acronyms**

- **BMS** = bare-metal stent
- **BrdU** = bromodeoxyuridine
- **EC** = endothelial cell
- **ELISA** = enzyme-linked immunosorbent assay
- **EPC** = endothelial progenitor cell
- **G-CSF** = granulocyte-colony stimulating factor
- **PDGF** = platelet-derived growth factor
- **PES** = paclitaxel-eluting stent
- **SEM** = scanning electron microscopy
- **SMA** = smooth muscle actin
- **SMC** = (vascular) smooth muscle cell
- **SPC** = smooth muscle progenitor cell
- **VEGF** = vascular endothelial growth factor
- **VPC** = vascular progenitor cell

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![Figure 1](image-url)
mm, Boston Scientific, Natick, Massachusetts; PES = TAXUS, 2.75 mm × 16 mm, Boston Scientific) One day after stenting, rabbits were randomized to receive either recombinant human G-CSF (n = 10) or placebo (n = 10) for 6 days. At day 30, selected stents were dissected longitudinally to evaluate luminal surface as en-face, fixed in glutaraldehyde, and processed for scanning electron microscopy (SEM) for evaluation of re-endothelialization (n = 3 each for four groups, BMS vs. PES in G-CSF vs. placebo). We previously validated that SEM and traditional immunohistochemical staining with endothelial cell marker CD31, PECAM-1 was well correlated in terms of re-endothelialization (11). At day 60 (n = 7 each for four groups, BMS vs. PES in G-CSF vs. placebo), stents were fixed in 10% buffered formalin with perfusion fixation. Plastic embedded sections were stained with hematoxylin and eosin, and the degree of neointimal growth was analyzed. Morphometric analysis was performed with a computerized digital image-analysis system (Image Pro version 4.5, MediaCybernetics, Silver Spring, Maryland).

Cell culture and immunocytochemical staining: All experiments dealing with humans or human products were conducted with informed consent and approved by the institutional review board of Seoul National University Hospital.

HUMAN EPCS. Peripheral blood (50 cc) was obtained from healthy donors with informed consent. The mononuclear cells fraction was collected and cultured as previously described (12). Isolated mononuclear cells were resuspended in EGM-2 BulletKit system (EGM-2 MV, Clonetics, San Diego, California) consisting of endothelial basal medium, human epidermal growth factor, vascular endothelial growth factor (VEGF), human fibroblast growth factor-basic with heparin, insulin-like growth factor-1, ascorbic acid, heparin, and 5% fetal bovine serum.

HUMAN ARTERIAL ENDOTHELIAL CELLS (ECS) AND VASCULAR SMOOTH MUSCLE CELLS (SMCs). Human right gastroepiploic arteries were obtained from gastrectomy specimens with informed consent. The ECs and SMCs were isolated and cultured (12). The ECs were grown in the same media as EPCs, and SMCs were cultured in DMEM (GIBCO, Grand Island, New York) containing 10% fetal bovine serum.

RABBIT PERIPHERAL BLOOD-ORIGINATED CELLS. Peripheral blood (70 cc) mononuclear cells were isolated by Histopaque-1077 (Sigma, St. Louis, Missouri) density gradient centrifugation and resuspended in EGM-2 MV. Cells were subsequently cultured in either EGM-2 MV to induce and maintain endothelial phenotype or EGM-2 supplemented with 10% fetal bovine serum and platelet-derived growth factor (PDGF)-BB stimulation (10 ng/ml, R&D Systems, Minneapolis, Minnesota) to facilitate vascular smooth muscle phenotype. After 3 weeks in culture, morphological appearance and immunocytochemical staining were used to define phenotypes. To detect endothelial phenotype, we used primary antibodies against CD31 (DAKO, Carpinteria, California), which were detected with PE-labeled goat anti-mouse IgG, and antibodies against alpha-smooth muscle actin (SMA) (FITC-conjugated, Sigma) were used to detect SMCs.

Flow cytometry. Antigen analysis was performed on both peripheral blood mononuclear cells and cultured cells from rabbits treated with G-CSF or placebo. Single cell suspensions were analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, New Jersey). The FITC-conjugated anti–alpha-SMA (Abcam, Cambridge, United Kingdom), VE-Cadherin (BD PharMingen, San Diego, California), and CD31 (DAKO) with secondary detection with PE-conjugated immunoglobulin were used. Isotype-matched IgG was used as a control.

Cell proliferation assay. The EPCs, ECs, and SMCs (1 × 10⁶ cells) were seeded to each well of a 96-well plate with a final volume of 200 μl/well conditioned medium. To assess the anti-proliferative effects of paclitaxel (Bristol-Myers Squibb, New York, New York) on human vascular cells and mobilized cells at various doses (0.1 nmol/l to 1000 μmol/l), the proliferative activities of each cell type were assessed by enzyme-linked immunosorbent assays (ELISAs) for bromodeoxyuridine (BrdU) incorporation (Roche Diagnostics, Mannheim, Germany) after 24 and 72 hours as previously described (13).

Statistical analysis. All data are presented as mean ± SD. Continuous variables were compared by using the Student t test, and multiple comparisons were performed by analysis of variance (ANOVA) with Bonferroni’s correction using SPSS version 11.0 (SPSS Inc., Chicago, Illinois). A p value of <0.05 was considered statistically significant.

RESULTS

Effects of G-CSF on neointimal hyperplasia after stenting. Before vascular injury, serum total cholesterol levels were similar between the G-CSF and placebo groups after 2 weeks of 1% cholesterol diet (910 ± 272 mg/dl vs. 1,097 ± 368 mg/dl, p > 0.05). At day 60 after stenting of BMS or PES at iliac arteries, the rabbits were killed and neointimal growth was analyzed (Fig. 2). Morphometric analysis (Figs. 2C and 2D; n = 7 each for four groups, by ANOVA with Bonferroni’s correction) showed that neointimal formation at BMS in the iliac artery of rabbits who received G-CSF was greater than that of rabbits who received placebo (neointimal thickness: 0.26 ± 0.04 mm vs. 0.34 ± 0.04 mm for BMS with placebo vs. BMS with G-CSF, p = 0.015, n = 7). As expected, PES (0.14 ± 0.05 mm) in the rabbits that received placebo showed reduced neointimal growth compared with BMS (0.26 ± 0.04 mm) in the rabbits that received placebo (PES with placebo vs. BMS with placebo, p < 0.001). In the rabbits where stem cells were mobilized by G-CSF, there was still a tendency of neointimal overgrowth even at PES, which was not statistically significant (0.14 ± 0.05 mm vs. 0.19 ± 0.04 mm for PES with placebo vs. PES with G-CSF, p = 0.36).
In terms of efficacy, the neointimal formation at PES even after G-CSF was significantly smaller than that at BMS after placebo (neointimal thickness: 0.19 ± 0.04 mm vs. 0.26 ± 0.04 mm, p = 0.036; neointimal area: 1.62 ± 0.21 mm² vs. 2.05 ± 0.25 mm² for PES with G-CSF vs. BMS with placebo, p = 0.015).

**Mobilization of putative vascular progenitor cells by G-CSF and in vitro differentiation to SMC or EC.** After 6 days of G-CSF injection, total white blood cells significantly increased in the G-CSF group compared with placebo (total white blood cell count: 36,900 ± 22,328/µl vs. 7,114 ± 1,401/µl for G-CSF vs. placebo, p < 0.01). There was no significant difference in the number of red blood cells and platelets between the two groups (data not shown).

Peripheral mononuclear cells were isolated for flow cytometry and serial cultures (Fig. 3). Granulocyte-colony stimulating factor increased not only the endothelial progenitor cells (CD31+, VE-Cadherin+, CD34+, KDR+) but also the fraction of double-positive cells for VE-Cadherin+/alpha-SMA+ or CD31+/alpha-SMA+, suggesting increased mobilization of putative vascular progenitor cells (VPCs). In serial cultures of the mobilized peripheral mononuclear cells, immunocytochemical staining showed that the cells mobilized by G-CSF gave rise to both endothelial and smooth muscle lineage cells. After 21 days of culture with specific factors, VEGF stimulation induced these cells mainly to endothelial lineage cells (cobble stone shape and CD31+), whereas PDGF induced a part of them to smooth muscle lineage cells (“hill and valley” morphology and alpha-SMA+) in addition to double-positive cells (CD31+/alpha-SMA+) or endothelial lineage cells.

**Incorporation of mobilized peripheral mononuclear cells into neointima and differentiation to SMC.** Peripheral blood from a rabbit that received either G-CSF or placebo for 6 days was collected. Isolated mononuclear cells (final isolated cell number was 1 × 10⁸) were then tagged with Dil and infused systemically after balloon injury (Fig. 4). The segment of injured artery was harvested 14 days later. In the rabbits receiving peripheral blood mononuclear cells (not mobilized by G-CSF but treated with placebo), the infused cells were scantily observed in the media and they did not express the smooth muscle phenotype, alpha-SMA (Fig. 4A). This result suggests that peripheral blood mono-
nuclear cells at normal status do not have substantial numbers of VPCs that incorporate into neointima and differentiate to vascular SMC. However, in the rabbits receiving the mobilized mononuclear cells by G-CSF, the DiI positive, infused mononuclear cells, were observed in both the media (Fig. 4B) and the neointima (Fig. 4C), some expressing the smooth muscle phenotype, alpha-SMA, and some not expressing it (Fig. 4D). These findings suggest that at least a portion of the cells mobilized by G-CSF incorporated into both the media and neointima, differentiated into SMCs, and participated in neointimal growth.

Differential inhibitory effect of paclitaxel on proliferation of smooth muscle versus endothelial lineage cells. To elucidate the mechanism for the maintained inhibition of neointimal formation at PES even after stem cell mobilization by G-CSF, we studied the effect of paclitaxel on the proliferation of mobilized rabbit EPCs, rabbit smooth muscle progenitor cells (SPC), human EPCs, and human vascular cells with ELISA to measure BrdU incorporation (Fig. 5). In a dose-dependent manner, paclitaxel preferentially inhibited rabbit SPCs and human SMCs proliferation compared with rabbit and human EPCs as well as human EC. At the presumable concentration of paclitaxel at the vascular tissue deployed with PES (100 nmol/l to 1 µmol/l, presented by Boston Scientific), proliferation of rabbit SPCs and human SMCs were inhibited even at a lower concentration (100 nmol/l), whereas rabbit EPCs and human EPCs and ECs were not. Only a higher concentration of paclitaxel (1 µmol/l) decreased the proliferative activity of human ECs, whereas that of EPCs was maintained. In other words, the proliferation of EPCs from both rabbit and human samples were not affected at these low and high working concentrations of paclitaxel in contrast to SPCs, SMCs, and ECs.

Effects of G-CSF on re-endothelialization of PES. Observing the inhibitory effect of paclitaxel at a high working concentration on endothelial proliferation in vitro, we evaluated the pattern of endothelial recovery after PES deployment in vivo by comparing with that after BMS.

We evaluated re-endothelialization at BMS and PES by gross and scanning electron microscopy (SEM) at day 28, a relatively early time point (Fig. 6, n = 3 each for four groups). The BMS showed complete re-endothelialization in the placebo as well as G-CSF administered groups. The PES in rabbits receiving placebo, however, showed tissue hemorrhage around stent struts in gross examination (Fig. 6A). The delayed endothelial recovery was confirmed in these samples by SEM of the luminal surface of arteries (Fig. 6B). The denuded area was approximately 10% of luminal surface, especially around the stent struts, where high tissue concentration of paclitaxel permeates into the tissue. However, PES in rabbits receiving G-CSF, which would mobilize EPCs, showed accelerated endothelial healing without hemorrhage around stent struts or denuded surface.

DISCUSSION

The administration of G-CSF might have the potential to promote neointimal growth in the setting of vascular injury; however, the mechanism of such effects remains unclear. Here, we showed that G-CSF mobilizes not only endothelial progenitor cells but also putative VPCs to the peripheral blood, which could incorporate into neointima and differentiate to SMC, leading to neointimal overgrowth at BMS. Such a trend of cytokine-induced neointimal overgrowth was also observed at PES, but the magnitude of neointimal overgrowth was reduced by PES, which might be associated with inhibition of smooth muscle lineage cell proliferation.
by paclitaxel. In terms of efficacy, the neointimal area at PES even after G-CSF treatment was still smaller than that at BMS after placebo. Under usual circumstances without stem cell mobilization, re-endothelialization at PES, especially near stent struts, was significantly delayed because paclitaxel at high working concentrations (especially near stent struts) inhibits the proliferation not only of SMC but also of EC. Stem cell mobilization with G-CSF, however, significantly accelerated endothelial recovery at PES. Such a unique finding was corroborated by the in vitro experiment, where paclitaxel preferentially inhibited the proliferation of smooth muscle lineage cells rather than endothelial lineage such as EPC.

**Putative VPCs in injured artery.** It has been generally believed that neointimal SMCs originate locally from the medial layer of the injured artery (7). But this traditional concept has been challenged by new evidence demonstrating other sources of SMCs in injured arteries (8,9). Recently, it has been reported in embryo (15), bone marrow (16), and peripheral blood (14) that putative VPCs might give rise to SMC. Our results support this new concept that the bone marrow might have the potential to give rise to VPCs and these progenitor cells might participate in vascular repair and lesion formation after injury. This study demonstrates for the first time that G-CSF could mobilize VPCs into the peripheral circulation and that these cells contribute to lesion growth as well as healing after vessel injury.

**Stem cell mobilization and neointimal growth.** Regarding restenosis, there are conflicting data and many controversies on the effect of stem cell mobilization, in both animal studies and human clinical trials. In a rat carotid balloon injury model with G-CSF, neointimal thickness was remarkably reduced by 60% (17). Previously, we reported, in a model of rabbit iliac artery balloon injury with intravas-
cular radiation therapy (11), that GM-CSF did not aggravate neointimal formation but accelerated re-endothelialization. In these two studies, the degrees or characteristics of vascular injury are different from those in the present study that evaluated stented artery in hypercholesterolemic rabbits. The rat carotid injury model represents "endothelial denudation" injury with simple and less atherogenic ("less SMC-dependent") milieu in rat species, and balloon injury with vascular radiation therapy implies total "eradication of local smooth muscle proliferative activity." In other words, in these two animal models, the rapidity of endothelial recovery rather than activity of SMC proliferation plays a pivotal role in determining neointimal formation.

In a clinical trial (5) and the present experimental study, neointimal thickness with BMS stenting increased after G-CSF mobilization. In these two studies, the nature of vascular injury is significantly different from that in previous experimental models. Coronary atherosclerotic lesions in patients are more complicated and rich in growth factors and cytokines stimulating SMC proliferation. Furthermore, stent deployment under the situation of hypercholesterolemia significantly stimulates SMC proliferation, resulting in neointimal formation at these lesions that are determined more by SMC rather than EC. Therefore, the cells mobilized by G-CSF, which includes not only endothelial but also smooth muscle progenitor cells, lead to more SMC proliferation than endothelial recovery, resulting in neointimal overgrowth. Taken together, the effect of stem cell mobilization by cytokines on neointimal growth might be variable among various vascular injury models.

**Re-endothelialization of drug-eluting stent.** Drug-eluting stents have been proven to be an effective therapeutic modality to prevent restenosis and have mostly replaced BMS in real-world practice. Despite the excellent results of PES in clinical trials (18), pathologic examinations of animal (19) and human (20) samples have shown that delayed re-endothelialization might be an important deleterious tissue reaction of PES (21). Experience from cyto-

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**Figure 5.** Differential inhibitory effect of paclitaxel on proliferation of smooth muscle versus endothelial lineage cells. (A) Anti-proliferative effects of paclitaxel on rabbit endothelial progenitor cells (EPCs) and smooth muscle progenitor cells (SPCs) were evaluated by enzyme-linked immunosorbent assays for bromodeoxyuridine (BrdU) incorporation. At the presumable tissue concentration (100 nmol/l to 1 μmol/l) of paclitaxel at vessel with TAXUS stenting, EPC still maintained its proliferative potential, whereas SPCs did not. *EPC versus vascular progenitor cell, p < 0.001; †p = 0.002. (B) Human EPCs, mature endothelial cells (ECs), and vascular smooth muscle cells (SMCs) were also evaluated. Paclitaxel caused a dose-dependent inhibition of SMC growth even from a low therapeutic concentration (100 nmol/l). In contrast, EC growth was inhibited only at 1 μmol/l of paclitaxel, a high therapeutic concentration. But proliferative activity of EPCs was not inhibited in the range of therapeutic concentration eluted from TAXUS stent. *EC versus SMC, p = 0.001; †EPC versus EC, p = 0.036; #EC versus SMC, p = 0.002 by analysis of variance with Bonferroni's correction.
toxic intravascular radiation therapy (11,22) has shown that delayed endothelial recovery aggravates vascular inflammation and leads to thrombosis and catch-up restenosis.

In this study, delayed endothelial recovery and tissue hemorrhage around stent struts were observed in PES. This delayed endothelial recovery, however, was overcome by G-CSF–induced stem cell mobilization, which accelerated the re-endothelialization of PES. To better understand the effects of paclitaxel on the various cells mobilized by G-CSF, we studied the effect of paclitaxel at the presumable tissue concentration where PES, TAXUS (Boston Scientific) stent is deployed (100 nmol/l to 1 μmol/l, presented by Boston Scientific). At these doses, proliferation of SMCs was preferentially inhibited rather than ECs in a dose dependent manner. Proliferative activity of ECs was inhibited at the higher concentration (1 μmol/l), but proliferation of EPCs was not affected. Taken together, PES in the setting of G-CSF administration might inhibit SMC proliferation yet at the same time be re-endothelialized by EPCs mobilized from the bone marrow.

It should be noted that the present study and a recently published article (23) that evaluated the effect of sirolimus on progenitor cells without mobilizers and on wire-mediated vascular injury in a mouse model both raise the possibility of impeded endothelial recovery with these commercially available drug eluting stents. Intriguingly, sirolimus showed similar inhibitory effects on both SPCs and EPCs (23), but paclitaxel showed a differential dose-related effect on SPCs and EPCs as well as mature SMCs and ECs. This difference might be due to the characteristics of the particular drug coated on the stent.

**Study limitations.** There are several limitations to this study. First, we tested neointimal growth and endothelial recovery after vascular injury in a rabbit iliac artery model. This study was conducted with juvenile rabbits without preformed atherosclerotic plaque in arteries. Even if the

**Figure 6.** Effects of stem cell mobilization on endothelial recovery after BMS and PES stenting. Gross (A) and scanning electron microscopy (SEM) findings (B) of stented rabbit iliac artery at 28 days. (First row) Endothelium and neointima were fully formed after BMS. (Third row) Aggravation of neointimal growth by G-CSF–mediated stem cell mobilization was reflected as the decreased transparency of gross finding and the disappearance of stent strut silhouette on SEM. (Second row) In case of PES, the evidences of delayed endothelial healing was observed as hemorrhage around PES struts in gross finding and uncovered stent struts in SEM finding. (Fourth row) After stem cell mobilization with G-CSF, however, endothelial layers were covered with cobblestone-shaped endothelial cells without focal hemorrhage, suggesting the enhanced endothelial recovery even on PES (arrowheads = hemorrhage; arrows = stent struts not covered by endothelium, owing to delayed re-endothelialization). Other abbreviations as in Figure 1.
rabbids received a high-cholesterol diet, the plaque is generally thin, non-calcified, and primarily confined to the intima with high lipid content. Despite limitations and inability to predict efficacy in humans, the hypercholesterolemic rabbit iliac artery injury model has been considered an acceptable choice for proving critical hypotheses regarding putative mechanism of action of vascular intervention (24).

Second, we used recombinant human G-CSF in this study. Therefore, it remains to be clarified whether human-directed G-CSF might be applicable to animal models such as the rabbit model. Because the pharmacokinetics of the G-CSF in rabbits is largely unknown, the dosage and injection interval of G-CSF were based on human data. However, in rabbits after 6 days of injection, total white blood cells measured in peripheral blood increased significantly compared with placebo. This finding might reflect the therapeutic efficacy of human-directed bone marrow mobilizing cytokines in experimental animal models.

Finally, it is conceivable that G-CSF could have a direct local effect if impregnated in a stent or delivered locally besides systemic progenitor cell mobilization. The potential direct effect of G-CSF on vasculature needs further investigation.

Conclusions. Granulocyte-colony stimulating factor mobilization might contribute to neointimal growth as well as endothelial recovery through the recruitment of VPCs, which differentiate into both EC and SMC. Paclitaxel in vitro and PES in vivo (TAXUS stents) effectively inhibited proliferation and accumulation of smooth muscle lineage cells. At sites of high drug-elution, PES showed a potential deleterious effect on re-endothelialization through interference of EC growth, which was overcome by EPCs mobilized by G-CSF. Our data suggest that the combination of PES and stem cell mobilization might have complementary effects on each other, one modality covering up the pitfalls of the other modality: DES suppressing neointimal overgrowth while stem cell mobilization enhances endothelial recovery on DES. Therefore, drug-eluting stents might be the optimal modality for revascularization in cytokine-based stem cell therapy.

Reprint requests and correspondence: Dr. Hyo-Soo Kim, Department of Internal Medicine, Seoul National University College of Medicine, 28 Yongon-dong Chongno-gu, Seoul 110-744, Korea. E-mail: hyosoo@snu.ac.kr.

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