Molecular Basis of Restenosis and Novel Issues of Drug-Eluting Stents

Teruo Inoue, MD; Koichi Node, MD

Restenosis after stent deployment is an overreaction of the wound healing response after vascular injury, and is characterized by the sequence of inflammation, granulation, extracellular matrix remodeling, and smooth muscle cell (SMC) proliferation and migration. In contrast, reendothelialization of at least part of the injured vessel surface, which is essential in the wound healing process, may occur at the site of stenting. Recent advances in drug-eluting stents (DES) have substantially reduced restenosis, but do not contribute to improve long-term prognosis, compared with bare metal stents (BMS). One of the reasons may be that reendothelialization is impaired after DES stenting. Regenerated endothelial cells and proliferated SMCs in the neointima are both in part derived from their progenitor cells, which are mobilized from bone marrow to injured vessel sites and differentiate into both vascular endothelial cells and SMCs. DES inhibits mobilization and differentiation of endothelial and smooth muscle progenitor cells, and thus not only inhibits restenosis but also impairs reendothelialization, which may lead to late stent thrombosis. To improve long-term prognosis in the DES era, adjunctive medical treatments inducing early reendothelialization, but inhibiting SMC proliferation, would be required. (Circ J 2009; 73: 615–621)

Key Words: Drug-eluting stents; Reendothelialization; Restenosis; Vascular repair

Percutaneous coronary intervention (PCI) is now an established treatment for coronary artery disease, but has long been compromised by the major limitation of restenosis and great efforts have been made to resolve this vexing problem. Recent advances in drug-eluting stent (DES) technology to reduce restenosis are among the great success stories in cardiology. In fact, DES such as sirolimus-eluting stents (SES) or paclitaxel-eluting stents (PES) have substantially reduced angiographic and clinical restenosis across broad lesion and patient subsets. Nevertheless, the current DES still can never be wholly suitable for all of the patients with coronary artery disease. Certain anatomic and clinical scenarios, such as patients with diabetes mellitus, restenotic lesions after DES, bypass graft disease, and bifurcations, continue to be problematic for restenosis reduction after DES stenting. In addition, DES do not contribute to an improvement of long-term prognosis.

A new concern beyond restenosis has arisen regarding the potential for late thromboses or very late thromboses after DES implantation, which limits discontinuation of the post-stent antplatelet regimen. These are rare, but life-threatening, complications that should never be neglected and are in part caused by impaired endothelial regeneration; namely, reendothelialization, which is essential in the normal wound healing process of injured vessels and is affected by the drugs that coat the stent’s surface. Sirolimus and paclitaxel are potent anti-mitotic agents that strongly inhibit smooth muscle proliferation and matrix growth, preventing neointimal formation and restenosis. However, delayed vessel wall healing because of impaired reendothelialization after DES stenting may go hand-in-hand with neointimal suppression. In addition, recent evidence suggests that DES also impair endothelial function in the segment distal to the stented site. Endothelial dysfunction has been also found in the microvasculature of the DES-implanted coronary artery. Anatomical, as well as functional, endothelial impairment may be in part associated with the evidence that DES never contribute to an improvement of long-term prognosis. We need to revisit both the pathophysiology of vascular injury and repair after PCI and the molecular basis of restenosis.

Stent-Induced Inflammatory Response

The response to PCI-induced vascular injury is characterized by the sequence of inflammation, granulation, extracellular matrix remodeling, and smooth muscle cell (SMC) proliferation and migration, which leads to neointimal thickening and restenosis. Stent placement is accompanied by stretching of the entire artery, de-endothelialization and compression of plaque, which often results in dissection of the tunica media and, occasionally, dissection of the adventitia. Such events induce a substantial local inflammatory reaction in the injured vessel wall, which is followed by proliferation of vascular components such as SMCs and extracellular matrix, leading to neointimal thickening and restenosis. In contrast, reendothelialization of at least part of the injured vessel surface may occur at the site of stenting.

In the early stage of the inflammatory process after vascular injury, activated leukocytes, neutrophils as well as monocytes, and platelets play an important role. A layer of platelets and fibrin are first deposited on the injured and de-endothelialized vessel surface. A sequential adhesion model of leukocyte attachment to and transmigration across
Surface-adherent platelets have been proposed as a mediator of platelet activation on the injured vessel surface. Activated platelets on the injured vessel surface express adhesion molecules, such as P-selectin, that was released from α-granules. The initial tethering and rolling of leukocytes on platelets are mediated by P-selectin binding to leukocyte receptors, such as P-selectin glycoprotein ligand-1 (PSGL-1)22-24. Leukocytes then adhere firmly to the vessel surface through leukocyte integrin Mac-1 (CD11b/CD18) via direct attachment to platelet receptors such as glycoprotein Ibα (GPIbα)25 and through cross-linking with fibrinogen to the GPIIb/IIIa receptor (Figure 1)26. These inflammatory processes are followed by proliferation of vascular components, such as SMCs and extracellular matrix, leading to neointimal thickening27. Mac-1 is thought to be an important signaling protein in the mechanism of restenosis. Monoclonal antibody blockade28 and the absence of Mac-129 reduces neointimal thickening after experimental angioplasty and stenting. We previously demonstrated, using the flow cytometric analysis, that Mac-1 is activated and upregulated on the surface of neutrophils in the coronary circulation, time-dependently after PCI, with the maximum response at 48 h. That process was accompanied by a sustained increase in the expression of P-selectin on the surface of platelets, being related to increased angiographic late lumen loss and restenosis30-33. Our results provided in-vivo human data supporting the hypothesis of the experimental works that platelets deposited at the site of injury are capable of local leukocyte integrin activation.

**SMC Proliferation and the Cell Cycle**

Under normal conditions, vascular SMCs are quiescent and exhibit low levels of proliferative activity. PCI-induced mechanical vascular injury and the subsequent inflammatory response trigger SMC proliferation through the G1/S transition of the cell cycle34,35. The different phases of the cell cycle are regulated by a series of protein complexes comprising cyclins, cyclin-dependent kinases (CDKs) and their cyclin-dependent kinase inhibitors (CKIs). CKIs, such as p27Kip1 or p21Cip1, regulate the G1/S transition through binding to cyclins E/CDK2 and inhibiting CDK2 activity, leading to cell cycle arrest. After arterial injury produces downregulation of p27Kip1, which triggers an increase in cell proliferation, p27Kip1 is upregulated together with p21Cip1 in later phases of the arterial healing response and is associated with a significant decline in cell proliferation and an increase in procollagen and transforming growth factor-β synthesis.36 Those findings suggest that p27Kip1 and p21Cip1 are endogenous regulators of G1 transit in vascular SMCs and inhibit cell proliferation after arterial injury. Overexpression of p27Kip1 results in cell cycle arrest in the G1 phase.36 Gene transfer of p27Kip1 or p21Cip1 into balloon-injured arteries produces a significant reduction in SMC proliferation and neointimal thickening.34,37,38 Conversely, inhibition of p27Kip1 increases the number of cells in the S phase. p27Kip1-deficient mice develop hyperplasia in multiple organs, including endocrine tissues, thymus, and spleen.39-41 Importantly, deficiency of p27Kip1 results in a prominent vascular phenotype with markedly increased neointimal thickening and inflammatory cell accumulation after mechanical arterial injury.42 The level of p27Kip1 is also regulated by constituents of the extracellular matrix. Mature collagen, such as polymerized type 1 collagen, has been shown to increase the levels of p27Kip1. In addition to their proliferation, migration of SMCs is also regulated by the cell cycle.32 SMCs in G1, but not in later phases of the cell cycle, are able to migrate on mitogenic stimuli, but upregulation of p27Kip1 inhibits cellular migration.44,45 The cell cycle is the common hub of the different phases of the restenosis process.

**Anti-Restenotic Mechanism of DES**

Clinical experience with systemically administered drugs,
such as antiplatelet agents, anticoagulants, calcium-channel blockers, and angiotensin-converting enzyme inhibitors, has been almost negative for the prevention of restenosis, even though these agents were found to be beneficial in animal models. The lack of efficacy in human studies may be in part related to an insufficient concentration of the drug at the injury site or to a lack of chronic dosing. Similarly, oral administration of immunosuppressive or anti-mitotic agents has also failed to show any benefit, and, in fact, there was a higher incidence of adverse side-effects because of their cytotoxicity. The unprecedented clinical success of recent DES (SES or PES) technology (ie, stent-based local release of sirolimus or paclitaxel at the site of vascular injury via polymer coated stents) depends on achieving effective local drug concentrations for a designated period, avoiding systemic toxicity.

Sirolimus (rapamycin) is an immunosuppressive agent and a pro-drug that binds to specific cytosolic proteins. It binds to the immunophilin FK506-binding protein 12 (FKBP12), which is upregulated in human neointimal SMCs. The FKBP12/rapamycin complex binds to a specific cell-cycle-regulatory protein, the mammalian target of rapamycin (mTOR), and inhibits its activation. mTOR is involved in a crucial event of the cell cycle, the transition between the G1 and S phases, in which DNA replication occurs, thus leading to irreversible cellular commitment toward division. Thus, sirolimus has a cytostatic effect and can induce cell cycle arrest in late G1. A known effect of sirolimus is inhibition of the serine/tyrosine kinase p70S6K and inhibition of collagen synthesis involved in extracellular matrix formation and smooth muscle cell migration. In addition, sirolimus also inhibits the collagen synthesis involved in extracellular matrix formation and smooth muscle cell migration. In addition, sirolimus also attenuates the inflammatory reaction in the vessel wall by inhibiting inflammatory cell activation.

Vascular Repair After Stent Implantation

Recent exciting studies suggest that endothelial progenitor cells (EPCs) mobilized from bone marrow into peripheral blood contribute to endothelial regeneration and postnatal neovascularization, leading to angiogenesis or vasculogenesis in ischemic organs. In addition, regenerated endothelial cells differentiated from bone marrow-derived EPCs can also contribute to neovascularization as part of the process of neointimal hyperplasia. Evidence in support of these hypotheses is demonstrated by a vascular injury model in bone-marrow-chimeric mice and DNA in situ hybridization for the human Y chromosome in the sex-mismatched heart transplantation model. Under stimulation by vascular injury such as stenting, bone-marrow-derived stem cells, which include vascular progenitor cells as both EPCs and SMPCs, are also mobilized from bone marrow into peripheral blood contribute to endothelial regeneration and postnatal neovascularization, leading to angiogenesis or vasculogenesis in ischemic organs.
alization and vascular SMCs that may lead to neointimal thickening and restenosis (Figure 3).

We recently observed serial changes in the number of circulating CD34-positive bone-marrow-derived stem cells, as well as activation of Mac-1 on the surface of neutrophils in patients undergoing elective PCI with BMS or SES. BMS deployment was accompanied by activation of Mac-1 across the coronary artery bed and was highest in patients with a bare metal stent (BMS) who subsequently developed restenosis, was intermediate in BMS patients without restenosis, and lowest in patients receiving a sirolimus-eluting stent (SES). The number of CD34-positive cells was also highest in BMS patients with restenosis, intermediate in BMS patients without restenosis, and lowest in patients with a SES (Figure 4). Accumulation of CD34-positive cells in the peripheral blood peaked at 7 days and correlated with the extent of transcardiac Mac-1 activation at 48 h and, importantly, with late lumen loss within the stent at follow-up angiography. The number of CD34-positive cells was highest in BMS patients with restenosis, intermediate in BMS patients without restenosis, and lowest in SES patients (Figure 4). Among the CD34-positive bone-marrow-derived cells, smooth muscle precursor cells in peripheral blood were increased in BMS patients with restenosis, but not in

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**Figure 3.** Bone-derived stem cells participate in neointimal smooth muscle cell proliferation and reendothelialization. Both proliferated smooth muscle cells and regenerated endothelial cells are in part derived from bone-derived stem cells (ie, endothelial and smooth muscle progenitor cells).

**Figure 4.** Change in activated Mac-1 demonstrated as antigen binding capacity for 8B2, an antibody for the activation-dependent epitope of Mac-1, at 48 h (Left panel) and the change in the number of circulating CD34-positive bone-marrow-derived stem cells on day 7 (Right panel) after coronary stent deployment. Mac-1 activation was highest in patients with a bare metal stent (BMS) who subsequently developed restenosis, was intermediate in BMS patients without restenosis, and lowest in patients receiving a sirolimus-eluting stent (SES). The number of CD34-positive cells was also highest in BMS patients with restenosis, intermediate in BMS patients without restenosis, and lowest in patients with a SES.
BMS patients without restenosis or in SES patients. Most interestingly, EPCs were increased after deployment of BMS, but markedly reduced after deployment of SES. These observations indicate that stent deployment itself sets off a cascade of biological events that act not only locally but also likely at a distance from the site. Stent-induced inflammation promotes the release of stem cells into the peripheral blood, which participate in the healing response of the injured blood vessel, possibly contributing SMPCs to the developing neointima and EPCs to the reendothelialization of the denuded surface, although the precise mediators responsible for stem cell recruitment remain to be defined.

Actually, transdifferentiation of the vascular progenitor cells to an endothelial cell lineage or a SMC lineage is theoretically possible, but another possibility, such as local paracrine effects of accumulating bone-marrow-derived stem cells on endothelial regeneration or vascular SMC proliferation and migration, is more likely to be operating. In any case, our results lead to the important suggestion that excessive mobilization of stem cells may lead to restenosis and that its absence may impair reendothelialization.

Future Aspects of the PCI Strategy in the DES Era

The molecular and cellular mechanisms of inflammation and cellular proliferation in vascular injury and repair indicate that DES implantation may be a double-edged sword. In the repair of injured vessels, overreaction of the wound healing response leads to restenosis, but on the other hand, reendothelialization is essential for normal wound healing. DES inhibit not only the overreaction and restenosis but also reendothelialization and normal wound healing (Figure 5).

Beyond sirolimus, various immunosuppressive agents are going to be used in DES technology. Compared with sirolimus, these agents seem to have less inhibitory effects on SMC proliferation, and ongoing early clinical trials of DES with these agents on restenosis reduction had results that were not comparable with those for SES. However, these drugs are expected to impair reendothelialization less.

To improve reendothelialization at stented sites, an EPC-capturing stent has been devised and evaluated in a preclinical situation. Now we should reconsider potential strategies in the DES era. First, we should make proper use of various stents, based on an evolving understanding of the pharmacological or biological properties of these stents. Second, adjunctive medical treatment with statins, angiotensin-receptor blockers, thiazolidinediones or other drugs that potentially induce reendothelialization would be essential, especially after SES-stenting. These treatments should simultaneously be aimed toward more strict interventions for risk factors such as hypertension, diabetes and dyslipidemia that substantially limit the long-term prognosis of patients undergoing PCI. Finally, at present PCI does not reduce the risk of death, myocardial infarction or other major cardiovascular events when added to optimal medical therapy. Therefore, interventional cardiologists would do well to heed that appropriate late loss is required for normal wound healing in the stented vessel’s wall, so far as clinical restenosis can be avoided, and that the real endpoint of PCI is not restenosis prevention but improvement of the long-term mortality and morbidity of the patient.

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