Possible Role of Damaged Neoendothelial Cells in the Genesis of Coronary Stent Thrombus in Chronic Phase

A Dye-Staining Angioscopic Study

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Summary

The mechanism(s) underlying formation of coronary stent thrombus (ST) in chronic phase is yet unclear. Endothelial cells are highly antithrombotic, therefore, it is conceivable that neoendothelial cells (NECs) covering stent struts are damaged and cause ST. This study was performed to examine the role of damaged NECs covering coronary stent struts in the genesis of occlusive or nonocclusive ST in chronic phase.

(1) Forty-four patients with acute coronary syndrome (17 females and 27 males) underwent dye-staining coronary angioscopy, using Evans blue which selectively stains damaged endothelial cells, 6 months after bare-metal stent (BMS) deployment. Neointimal coverage was classified into not covered (grade 0), covered by a thin layer (grade 1), and buried under neointima (grade 2) groups. (2) In 7 beagles, the relationships between neointimal thickness and ST were examined 6 months after BMS deployment. (3) The NECs on the struts were stained blue in 4 of 25 patients with grade 2 and in 11 of 20 patients with grade 0/1 (P < 0.05). ST was observed in none of the former and in 5 of the latter (P < 0.05). (4) In beagles, neointimal coverage was grade 0/1 when neointimal thickness was 80.2 ± 40.0 μm, whereas grade 2 when thickness was 184 ± 59.4 μm. ST was observed in 9 of 15 struts with neointimal thickness within 100 μm and in one of 17 struts with thickness over 100 μm (P < 0.05). ST arose from damaged NECs covering the stent struts. NECs may have been damaged due to friction between them and struts due to thin interposed neointima which might have acted as a cushion, resulting in ST. (Int Heart J 2011; 52: 12-16)

Key words: Stent thrombus, Neointimal thickness, Neoendothelial cell damage, Evans blue dye, Dye-staining angioscopy

Coronary stent deployment has contributed to the treatment of coronary artery disease. However, concerns have been raised regarding the risk of late coronary thrombosis, ie, acute coronary syndrome caused by an occlusive coronary stent thrombus in chronic phase, which requires a prolonged dual antiplatelet therapy and places an economic burden on patients.

Stent thrombus (ST), both occlusive and nonocclusive, was observed by angioscopy in 8-29% of patients after bare-metal stents (BMS), and in 19-86% after drug-eluting stents (DES) at 6 months.13 Inappropriate coverage of the stent struts by the neointima has been attributed to a higher incidence of ST.

The mechanism(s) underlying ST is yet unclear. Endothelial cells are highly antithrombotic. Therefore, it is conceivable that neoendothelial cells (NECs) covering stent struts are damaged and cause ST.

The present study was performed to examine the relationships among neointimal thickness, NEC damage, and ST in cases of BMS in patients and animals in chronic phase.

METHODS

Subjects: From April 1, 1999 to March 31, 2008, 44 consecutive patients with acute coronary syndrome (17 females and 27 males; mean ± SD age, 61.0 ± 8.0 years; 19 with unstable angina, 7 with non-ST elevation myocardial infarction, and 18 with ST-elevation myocardial infarction) in whom a single BMS (Multilink in 21, S 60 in 7, PS in 2, and Liberty in 14 patients) was deployed into the culprit lesion (left anterior descending artery in 24, left circumflex artery in 9, and right coronary artery in 11 patients) underwent conventional angioscopy and dye-staining angioscopy of the stented coronary arteries 6 months after stent deployment.

Coronary stent deployment and angioscopy were performed at Toho University Sakura Hospital (Sakura, Japan) and Funabashi-Futawa Hospital (Funabashi, Japan) with the approval of their Institutional Review Boards. All patients in the present study provided informed consent with regard to all the procedures described herein.

Angioscopy system: The angioscopy system was composed of a light source (OTV-A, Olympus Corporation, Tokyo), a 5-F angioscope (VecMover, Clinical Supply Company, Gifu, Japan), and a 5-F angioplasty catheter. The angioscope system was composed of a light source (OTV-A, Olympus Corporation, Tokyo), a 5-F angioscope (VecMover, Clinical Supply Company, Gifu, Japan), and a 5-F angioplasty catheter.
pan), and a color chilled charge-coupled device (CCD) camera (CSVEC-10, Clinical Supply). Before carrying out the angioscopy examination, the white balance of the camera was adjusted for color correction. The system has been approved for clinical use by the Japanese Ministry of Health, Labor and Welfare. Details of this system are described elsewhere. Conventional angioscopy: All patients received heparin (5000 IU i.v.) just before the procedure and nitroglycerin (200 μg, i.c.) just before coronary angiography.

The diameter stenosis of the stented coronary artery was measured with a TCS Symphony 2.02 (McKesson Co, North Charleston, SC, USA) using the angiograms, and was expressed as a percentage. After coronary angiography, an angioscope was introduced into the stented coronary artery not guided by a guidewire to prevent damage to the NEC by the guidewire. The fiberscope incorporated into the angioscope was advanced into the stented coronary segment so as to locate the fiberscope tip at the proximal end of the stent. The balloon of the angioscope was inflated to stop the blood flow therein and the luminal surface was observed by advancing the fiberscope while displacing the blood by infusion of heparinized saline solution (10 IU/mL) at a rate of 2 mL/s for 10 to 20 seconds through the flush channel of the angioscope.

To accurately confirm the location of the fiberscope tip (and accordingly the observed portion), the angioscopic and fluoroscopic images were displayed simultaneously on a television monitor.

The degree of neointimal coverage was classified as grade 0 when stent struts reflected light because there was no neointimal coverage (similar to immediately after deployment); grade 1 when stent struts were covered by neointima but still visible; and grade 2 when stent struts were not visible because they were buried in the neointima. Dye-staining angioscopy: Evans blue is a blue dye which was used clinically for cardiac output measurement many years ago. Since 1995, this dye has been clinically administered into the arterial system for selective staining of damaged vascular endothelial cells and fibrin without obvious side effects. Beneficial effects of this dye in preventing coronary restenosis have been reported.

After observation by conventional angioscopy, one mL of 2.5% Evans blue solution was injected during balloon inflation into the artery through the flush channel of the angioscope to stain the damaged NECs, and then the balloon was deflated for blood flow restoration. One to 2 minutes later, the balloon was inflated again and the coronary luminal surface was observed by angioscopy. The details are described elsewhere. NECs were considered to have been damaged when they were stained blue with the dye.

Animal experiments: Animal experiments were carried out at the Institute for Animal Experiments of the Jikei University School of Medicine (Tokyo). The protocols were approved by the Jikei University Administrative Panel on Laboratory Animal Care.

Seven beagles were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). After administration of heparin (500 U/kg, i.v.), an 8-F guiding catheter specially designed for beagle use was introduced through the right common carotid artery into the left coronary artery. Next, a BMS (3 Multilink and 4 S60 stents) was deployed into the left anterior descending coronary artery. After recovery from anesthesia, the beagles were cared for according to the guidelines of the university. Six months later, the beagles were sacrificed by administration of pentobarbital sodium (60 mg/kg, i.v.) and potassium chloride (100 mg/kg, i.v.) according to the study protocol; the stented coronary segments were excised, perfused with saline solution, and observed by angioscopy to determine the location of the struts and grade of neointimal coverage. Since the light of the angioscope was seen through the wall, the location of the struts could be determined. The stented coronary segment was then fixed with 10% formaldehyde solution, cut into slices 10 μm in thickness at the center of the stent, the slices stained by Azan staining, and the relationships between the ST formation and the neointima thickness were examined by microscopy using a slice from each beagle.

Statistical analysis: The data were tested using Fisher’s exact test. A P < 0.05 was considered significant.

RESULTS

Representative examples: Panels A and B of Figure 1 show angioscopic images before and after dye-staining of a coronary segment in which a BMS was deployed in a patient with ST-elevation myocardial infarction. In this patient, neointimal coverage was grade 2 and NECs were not stained blue with Evans blue, indicating that the NECs were not damaged.

Panels A and B of Figure 2 show angioscopic images before and after dye-staining of a stented coronary segment in a patient with unstable angina. Neointimal coverage was grade 1 and the NECs on the struts but not those in the neighboring portions were stained blue, indicating damages were localized to the NECs on the struts.

Panels of A and B of Figure 3 show angioscopic images before and after dye-staining in a patient with ST-elevation myocardial infarction. In this patient, neointimal coverage was grade 1, and the NECs not only on the struts but also those in the neighboring portions were diffusely stained, indicating extensive NEC damage.

Panels A and B of Figure 4 show angioscopic images of a patient with ST-elevation myocardial infarction before and after dye-staining in a patient with unstable angina. In this patient, neointimal coverage was grade 1, and the NECs not only on the struts but also those in the neighboring portions were diffusely stained, indicating extensive NEC damage.

Figure 1. Staining of neoendothelial cells (NECs) covering stent struts with Evans blue 6 months after bare-metal stent (BMS) deployment. A and B: A 62 year-old-male with ST-elevation myocardial infarction. A multilink stent was deployed into the anterior descending coronary artery. The stent struts were not visible and therefore neointimal coverage was grade 2. The NECs covering the struts were not stained with Evans blue, indicating that they were not damaged.
ter dye-staining, respectively. Neointimal coverage was grade 0 because the struts reflected light and red thrombus arose from the struts. The NECs on the struts and in the neighboring

Figure 2. Staining of neoendothelial cells (NECs) covering stent struts with Evans blue 6 months after bare-metal stent (BMS) deployment. A and B: A 58 year-old-male with unstable angina. The struts of a stent were covered by neointima but they were visible and therefore neointimal coverage was judged to be grade 1 (arrows in A). The NECs covering the struts (arrow in B) but not in the adjacent portions were stained with Evans blue, indicating that damage was confined to the NECs on the struts. The struts indicated by the arrow in A correspond to the arrow in B.

Figure 3. Staining of neoendothelial cells (NECs) 6 months after bare-metal stent (BMS) deployment. A and B: A 65-year-old male with ST-elevation myocardial infarction. The struts of a stent were covered by neointima but were visible and therefore neointimal coverage was judged to be grade 1 (arrow in A). The NECs not only on the struts (arrow in B) but also those in the neighboring portions were diffusely stained with Evans blue, indicating diffuse damage. The strut indicated by the arrow in A corresponds to that in B.

Figure 4. Staining of neoendothelial cells (NECs) 6 months after bare-metal stent (BMS) deployment. A and B: A 57 year-old-female with ST-elevation myocardial infarction. Red thrombi arose from the naked portion of the stent strut. Neointimal coverage was grade 0 because the struts reflected light (arrow in A). The strut and the adjacent portions were diffusely stained with Evans blue, indicating diffuse NEC damage.

Figure 5. Occlusive stent thrombus (late stent thrombosis) formed 7 months after bare-metal stent (BMS) deployment. A and B: A 57 year-old-male in whom ST-elevation myocardial infarction developed 7 months after BMS deployment. Arrow in A: naked (grade 0) strut. Arrowheads: occlusive red thrombi. Arrow in B: struts and neighboring portions stained blue. Arrowheads correspond to those in A.

Figure 6. Neoendothelial damage and stent thrombosis (ST). Left: Relationships between grading of stent coverage and NEC damage patterns. NEC damage visualized by dye-staining angioscopy was more frequently observed in the grade 0/1 group than in the grade 2 group. Right: Relationships between patterns of NEC damage and ST. ST was observed more frequently in the diffusely damaged NEC group than in the localized damage group. * P < 0.05.

Panels A and B of Figure 5 show occlusive thrombi formed on grade 0 struts in a patient in whom ST-elevation myocardial infarction developed 7 months after a BMS deployment. The struts and the neighboring portions were diffusely stained blue.

Relationship between NEC damage and grading of neointimal coverage: Damaged NECs visualized by Evans blue were observed in 13.3% of the grade 2 group and in 80% of the grade 0/1 group (Figure 6).

Relationships between stent thrombus (ST) and patterns of NEC damage: ST was observed in the diffusely damaged NEC group but not in the localized NEC damage group (Figure 6).

Animal experiments: The relationships between neointimal thickness and the angioscopic images of the stent struts were examined in stent struts without ST because ST may have disturbed the strut imaging. Neointimal coverage of stent struts
**Discussion**

Neointimal formation after stent implantation is a wound healing response after vascular injury, characterized by inflammation, granulation, extramatrix remodeling, progenitor cell migration and differentiation, and smooth muscle cell proliferation and migration and their consequent change into connective tissues. However, the exact mechanism(s) underlying ST in chronic phase remains obscure. Endothelial cells are highly antithrombotic. The same may also be true for NECs regenerated after stent implantation. Therefore, the present study focused on whether or not NECs were damaged.

In the present study, NECs covering the stent struts were frequently damaged in patients having grade 0 or 1 neointimal coverage of the stent struts. Neointima is mainly composed of newly formed collagen fibers and is located between neointimal cells and stent struts, and acts as a cushion between NECs and struts. Because of the stiff nature of stent struts, NECs may be damaged by friction between them and struts due to thin interposed neointima which might have acted as a cushion.

Types of NEC damage were classified into localized and diffuse types. ST was observed in the latter type. Diffuse NEC damage may have enhanced ST formation and allowed attachment of ST to the damaged NECs. What factor (or factors) determined these two different patterns of NEC damage has yet to be elucidated.

By comparing angioscopic and optical coherence tomo-graphic images in patients, Tsujimoto observed that stent struts were visible (grade 0 or 1) by angioscopy when the neointimal thickness was within 130 μm by optical coherence tomography.

The ST was observed in grade 0 or 1 group of patients in the present study. In animals, the struts were visible (grade 0 or 1) when the neointimal thickness was around 85 μm and ST was frequently observed when the neointimal thickness was within 100 μm. All these findings indicated that NECs were damaged and ST was formed on the struts when neointimal thickness was within approximately 100 μm.

Fresh ST was observed in both patients and animals at 6 months after stent deployment in this study. It is conceivable that even if the NECs were regenerated repeatedly, they were damaged repeatedly by friction between them and the struts due to luminal blood pressure changes and cardiac motion, thus fresh thrombus was formed repeatedly even at 6 months (Figure 8). Exactly how to terminate this “vicious cycle” is an essential requisite for effective prophylactic treatment of ST, at least in the case of BMS.

ST was observed when neointimal coverage was grade 0 or 1 in the case of sirolimus and zulorimus-eluting stents, as in the case of ST formation in the present study. In addition to the effects of drugs and polymers on NECs, a similar mechanical mechanism may underlay ST in the DES era (Figure 8).

Based on the results obtained in the present clinical and animal studies, it is conceivable that to control neointimal regeneration over 100 μm, and below an appropriate thickness which does not cause significant restenosis is necessary to prevent ST, at least in the case of BMS.

Higo, et al observed that thrombus was more frequent in the yellow lesions than in the white lesions of stented lesions in the chronic stage of DES implantation. They suggested that newly formed yellow intima within the stented coronary segments is a potential risk factor of ST. Takayama, et al reported that yellow plaques within the stented lesions are risk factors for ST.

Whether or not neointimal thickness is a major determinant of ST in the DES era remains to be elucidated.

**Study limitations:** Neointimal thickness was not measured in a clinical setting in this study, thereby weakening our hypothesis that neointimal thickness within 100 μm is the major determining factor for ST.
Conclusion: NECs covering bare-metal coronary stent struts were damaged in patients having struts not covered or thinly covered by neointima. NEC damage was classified into localized damage or diffuse damage. ST was observed in the latter type. In animals, ST was observed when the neointima thickness was 100 μm or less. NECs may have been damaged by friction between them and the stent struts due to thin interposed neointima which might have acted as a cushion, resulting in the formation of ST.

REFERENCES