Drug-Eluting Stent and Coronary Thrombosis
Biological Mechanisms and Clinical Implications

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Abstract—Although rare, stent thrombosis remains a severe complication after stent implantation owing to its high morbidity and mortality. Since the introduction of drug-eluting stents (DES), most interventional centers have noted stent thrombosis up to 3 years after implantation, a complication rarely seen with bare-metal stents. Some data from large registries and meta-analyses of randomized trials indicate a higher risk for DES thrombosis, whereas others suggest an absence of such a risk. Several factors are associated with an increased risk of stent thrombosis, including the procedure itself (stent malapposition and/or underexpansion, number of implanted stents, stent length, persistent slow coronary blood flow, and dissections), patient and lesion characteristics, stent design, and premature cessation of antiplatelet drugs. Drugs released from DES exert distinct biological effects, such as activation of signal transduction pathways and inhibition of cell proliferation. As a result, although primarily aimed at preventing vascular smooth muscle cell proliferation and migration (ie, key factors in the development of restenosis), they also impact reendothelialization, which leads to delayed arterial healing, and induce tissue factor expression, which results in a prothrombogenic environment. In the same way, polymers used to load these drugs have been associated with DES thrombosis. Finally, DES impair endothelial function of the coronary artery distal to the stent, which potentially promotes the risk of ischemia and coronary occlusion. Although several reports raise the possibility of a substantially higher risk of stent thrombosis in DES, evidence remains inconclusive; as a consequence, both large-scale and long-term clinical trials, as well as further mechanistic studies, are needed. The present review focuses on the pathophysiologic mechanisms and pathological findings of stent thrombosis in DES. (Circulation. 2007;115:1051-1058.)

Key Words: stents ■ thrombosis ■ pathology ■ physiology ■ risk factors ■ arteries ■ myocardial infarction

With the introduction of balloon-expandable stents, coronary remodeling and, in turn, restenosis were reduced compared with angioplasty alone. With the risk of restenosis still in the range of 15% to 20%, however, drug-eluting stents (DES) designed to release pharmacological agents after deployment were developed to inhibit the response to injury mainly responsible for restenosis after bare-metal stent (BMS) implantation (ie, vascular smooth muscle cell migration and proliferation and proteoglycan deposition). As a result, restenosis and target-vessel revascularization could be reduced to rates below 10% after DES implantation.

In the first series of patients receiving BMS, stent thrombosis was already recognized as a severe complication after implantation owing to its high mortality. With the introduction of P2Y-receptor antagonists (ie, ticlopidine, clopidogrel) for platelet inhibition in combination with acetylsalicylic acid, the incidence of stent thrombosis decreased substantially in stable patients to levels as low as 1%. Most of the events occurred within the first 10 days after implantation; indeed, stent thrombosis after the first month was extremely rare with BMS.

Despite reduced restenosis rates, the frequency of in-stent thrombosis has not decreased with DES compared with BMS. Indeed, several hundred cases of stent thrombosis have been reported for rapamycin-coated stents. A number of reports imply that thrombosis rates of DES may even be higher in the “real world” than in clinical trials. Notably, many operators have experienced very late stent thrombosis (3 years after implantation and beyond) in a number of patients, which was not seen with BMS. This review will briefly discuss presently available clinical data and focus on pathophysiologic mechanisms of in-stent thrombosis in DES.

Clinical Evidence
Numerous reports describe the occurrence of acute (<24 hours), subacute (<30 days), late (>30 days), and very late (>12 months) stent thrombosis after DES implantation. However, the true incidence of stent thrombosis may be underestimated in clinical trials and could occur at substantially higher rates in the “real-world” setting, where more
Pathophysiology of In-Stent Thrombosis

Several factors that contribute to stent thrombosis have been recognized, which are discussed below.

Procedure-Related Factors

Among the procedure-related factors, smaller final lumen dimensions (stent malapposition and/or underexpansion), stent length, persistent slow coronary blood flow, placement of multiple stents, positive remodeling, dissections, geographic miss, and late stent malapposition due to thrombus resolution appear to be most important for the development of in-stent thrombosis.19–25 Also, in DES, stent length, stent underexpansion, and residual stenosis have been observed to correlate with an increased risk for stent thrombosis.26,27 These factors are of great interest because they can be avoided during the intervention, but they are unlikely to differ between BMS and DES.

Patient- and Lesion-Related Factors

Several patient-related factors have been associated with the development of in-stent thrombosis, including low ejection fraction,21 diabetes mellitus,28 advanced age,22 and stenting in the setting of an acute coronary syndrome.26 Similarly, in DES, primary stenting in acute myocardial infarction, diabetes mellitus, renal failure, and low ejection fraction appear to be associated with an increased risk for stent thrombosis.14,15,16,29,30 In particular, the increased risk in patients with acute coronary syndrome could be due to delayed healing, lack of endothelialization, and presence of a pronounced inflammatory and thrombogenic environment of the exposed necrotic core to flowing blood, accompanied by enhanced platelet reactivity; furthermore, rapamycin and paclitaxel potentiate thrombin-induced expression of tissue factor (see below).

Furthermore, certain lesion characteristics are reported to be associated with an increased risk of stent thrombosis. In DES, this pertains in particular to stenting of bifurcation lesions or in-stent restenosis lesions.14,29,30 In addition, interventional practice has changed, with advocates of “normal to normal” coronary artery stenting and revascularization of more complex lesions that carry a higher risk of stent thrombosis. This latter aspect makes comparisons of registries with historical controls difficult.

Antiplatelet Therapy

Stents are foreign bodies in the vessel wall and thus induce platelet adhesion and activation of the coagulation cascade. Furthermore, high-pressure implantation with noncompliant balloons induces significant vascular injury, with exposure of thrombogenic molecules of the subintima and media (including plaque material) to the blood stream. As a consequence, only potent platelet inhibition made the procedure feasible, and antiplatelet hyporesponsiveness has been associated with an increased risk for stent thrombosis.31 In line with this observation, discontinuation of antiplatelet therapy has been observed to be particularly associated with DES thrombosis.8,16,32 The appropriate duration of the long-term antiplatelet regimen for prevention of DES thrombosis remains to be assessed in randomized prospective trials; at present, a course of 12 months of dual-antiplatelet therapy may be considered especially in high-risk, real-world patients.

Thrombogenicity of the Stent

A predisposition for the development of stent thrombosis has been observed with certain stent materials; for example, platelet activation was greater during the 30 days after implantation of an open-cell versus a closed-cell stent.33 Stent strut thickness and polymer type and thickness also play an important role. It has been reported previously that the nonerodable polymers of the Cypher and Taxus DES provoke chronic eosinophilic infiltration of the arterial wall, suggestive of hypersensitivity reactions in a small number of cases.7,34 However, the causal relationship between polymer-induced inflammation and the incidence of late stent thrombosis has only been proven in a minority of patients possessing a proinflammatory phenotype. Detailed analysis of the morphological changes shows a localized immune response, with predominance of CD45-positive lymphocytes and eosinophils. In fact, our experience shows that all cases of hypersensitivity occur >4 months after DES implantation.7 The preclinical experience in a pig model also shows a progressive increase in the presence of granulomatous reactions, including eosinophilic infiltrate, starting at 28 days after Cypher stent implantation: 1 month, 14%; 3 months, 43%; 6 months, 60% (R.V., unpublished data). One possible explanation of these findings is that the hypersensitivity reaction peaks after the complete release of the drug and is likely related to the polymer. In addition, positive remodeling
has been observed in vessels showing a hypersensitivity reaction.

Furthermore, drugs loaded on DES may exert a prothrombogenic effect. Rapamycin (sirolimus), a macrocyclic lactone, is used on DES, because it is known to inhibit proliferation and migration of vascular smooth muscle cells, important factors in the development of neointima formation and restenosis, through interference with cell cycle regulators.35,36 On a subcellular level, rapamycin binds to the FK-binding protein 12 and subsequently inhibits the mammalian target of rapamycin. The mammalian target of rapamycin is a downstream target of the phosphatidylinositol-3 kinase pathway, which in turn is involved in an inhibitory fashion in the regulation of tissue factor in endothelial cells and monocytes.37–39 As a result, rapamycin inhibition of the mammalian target of rapamycin increases both thrombin- and tumor necrosis factor-α–induced endothelial tissue factor expression and activity at concentrations of rapamycin that are encountered in vivo (Figure 1).38,40

Paclitaxel is a lipophilic diterpenoid that binds to the β-subunit of the tubulin heterodimer, promoting tubulin polymerization, cell cycle arrest, and, eventually, inhibition of vascular smooth muscle cell migration and proliferation.41,42 In addition, paclitaxel is known to activate c-Jun NH2-terminal kinase,33,44 an important mediator of endothelial and monocyte tissue factor induction.7,39,45 Consequently, paclitaxel also enhances tissue factor expression and activity in endothelial cells;44 again, concentrations used in this in vitro study are comparable with local tissue concentrations of paclitaxel after stent deployment.46

In sirolimus-eluting stents, ≈80% of the rapamycin has eluted by 30 days, whereas paclitaxel-eluting stents have a biphasic drug release profile in vitro with an initial burst during the first 48 hours after implantation followed by a sustained low-level release for at least 2 weeks.47 However, both rapamycin and paclitaxel easily penetrate into cells of the vessel wall owing to their lipophilic properties, which leads to chronic retention of the drug in the arterial tissue.19,46,48 Thus, both rapamycin- and paclitaxel-induced tissue factor expression may contribute to a prothrombotic environment after deployment of DES, particularly in the acute and subacute setting and possibly in late stent thrombosis (Figure 1). The relationship between these findings and stent thrombosis in clinical practice requires further study, particularly to examine the degree and the spatiotemporal pattern of tissue factor expression in the arterial wall after deployment of DES.

**Impaired Reendothelialization**

Reendothelialization occurs after vascular injury and similarly after stent placement. Traditionally, it was believed that endothelial cells proliferate and migrate from intact neighboring coronary segments, eventually leading to the reendothelialization of the injured segment. In vitro, rapamycin and paclitaxel do not only inhibit proliferation and migration of vascular smooth muscle cells but equally suppress endothelial cells,7,35,36,38,49–51 thereby potentially impeding reendothelialization (Figure 2). We observed poor endothelial cell junction formation and microthrombi of focal platelet aggregation at 16 months after rapamycin stent implantation in a patient dying of a non-DES–related cause.52 It has been proposed that bone marrow–derived endothelial progenitor cells may also be involved in reendothelialization.53,54 Interestingly, rapamycin inhibits proliferation, migration, and differentiation of human endothelial progenitor cells in vitro.55,56 Hence, drugs loaded on DES may affect the number as well as the homing and proliferation of endothelial progenitor cells, thus further preventing proper endothelial healing (Figure 2).

In vivo, the time course of endothelial healing after stent implantation varies in different animal models. Although in healthy pigs, endothelialization is similar between BMS and DES at 28 days,48 a clear delay of endothelialization in both sirolimus- and paclitaxel-eluting stents was observed in a rabbit iliac-overlapping DES implantation model.46 In view of distinct differences with respect to vessel wall reactivity after stent implantation between animals and humans, however, these results cannot be applied entirely to the situation in humans. After BMS implantation, near-complete endothelialization has been suggested to occur by 3 to 4 months.6 A morphological autopsy study comparing coronary segments from patients after DES and BMS implantation revealed delayed arterial healing and poorer endothelialization after DES compared with BMS implantation of similar duration (Figure 3).7 Indeed, in 23 DES patients in that study, 14 had evidence of late-stent thrombosis, and of these 14 patients, 13 died of a DES-related cause.7 Thus, current evidence suggests delayed reendothelialization and arterial healing after implantation of DES compared with BMS, resulting in potentially
enhanced thrombogenicity (Figure 4). It is uncertain whether reendothelialization with DES is only delayed or persistently incomplete up to late time points. It is unclear in atherosclerotic human arteries how long it will take for DES to endothelialize. We have shown that in humans, delayed healing is common with current DES and that in those that thrombose, other factors, such as hypersensitivity reaction, bifurcating and ostial stenting, penetration of

Figure 2. DES reduce neointima formation but may increase stent thrombogenicity. Effect of sirolimus-eluting/paclitaxel-eluting stent strut on the local vessel wall after implantation. Sirolimus/paclitaxel reduces neointima formation by inhibiting vascular smooth muscle migration and proliferation (green arrows). However, the drugs also inhibit reendothelialization, induce tissue factor (TF), and may prevent homing and proliferation of endothelial progenitor cells (EPCs; red arrows/bars).

Figure 3. Delayed reendothelialization after DES implantation. Time course of arterial healing in BMS, Taxus DES, and Cypher DES from 1 to 8 months after stent implantation. Although some peristrut inflammation is observed in BMS at 1 month, complete arterial healing, including a well-established neointimal layer, is seen at 3 and 8 months’ duration. Taxus DES shows early fibrin deposition surrounding stent struts (*), which persists up to 8 months, as a sign of delayed healing. In contrast, Cypher DES shows predominance of inflammatory cells, including giant cell formation (black arrowheads), at early time points (1 and 3 months), whereas fibrin deposition is stronger at 8 months.
a necrotic core, stent malapposition, and restenosis, may also be important predictors of thrombosis.

There is compelling evidence that certain subsets of patients have a favorable long-term outcome after implantation of DES, which results in a reduced need for interventional or surgical revascularization due to sustained suppression of neointimal growth. Factors associated with greater healing from our experience include shorter stent length, less plaque area, less fibrin deposition, and greater endothelialization. Other factors that may influence healing are likely to be patient-related, such as antiplatelet therapy discontinuation, renal failure, diabetes mellitus, and a lower ejection fraction, which have all been reported in clinical studies.14 Furthermore, there is variability from patient to patient even in wound healing; the response to drug also varies, with some patients requiring a lower drug dose for equivalent benefit. Thus, many factors influence the healing process and vary with individual risk factors.

Risk Factors for Different Time Points of DES Thrombosis

Stent thrombosis may occur acutely (within 24 hours of stent placement), subacutely (up to 30 days after stent implantation), as late thrombosis (after 30 days), or as very late thrombosis (after 12 months). The most important risk factors for acute and subacute stent thrombosis are primary stenting in ST-segment elevation myocardial infarction and acute coronary syndromes.20,26 Additional risk factors include stent length, congestive heart failure, and a prothrombogenic state, such as metastatic cancer.19,20,26,27 One of the most significant risk factors for late and very late stent thrombosis appears to be discontinuation of antiplatelet therapy.26,27 Other predictors are stent underexpansion and residual reference segment stenosis.19,27

Design of Future DES

The stent coating influences thrombogenicity. Whether a simple chemical coating, such as titanium-nitride-oxide, that diminishes platelet adhesion and fibrinogen binding compared with stainless steel will be effective against restenosis and stent thrombosis remains to be elucidated in large clinical trials.57 Coating of stents with substances that potentially facilitate reendothelialization may represent a novel therapeutic approach. For example, in preliminary studies, coating of stents with CD34 antibodies designed to “capture” endothelial progenitor cells proved to accelerate endothelial coverage and appeared safe and feasible in humans.58 Similarly, stents loaded with an integrin-binding cyclic Arg-Gly-Asp peptide accelerated endothelialization by attracting endothelial progenitor cells in a porcine model.59 Further studies are needed to assess the long-term efficacy and safety of these biologically active stents. Furthermore, a combination of “prohealing” substances (such as vascular endothelial growth factor) with established “antirestenosis” drugs may represent an interesting approach to obtain the benefit of reduced restenosis without the cost of an increased risk for stent thrombosis.

Considering the delay in healing along with the unknown time of reendothelialization of current DES, prohealing strategies such as the use of peroxisome proliferator–activated receptor-γ agonists, which not only diminish inflammation but also enhance endothelialization, may also represent an interesting new approach for DES.60 Given the importance of tissue factor in the initiation of coagulation and thrombosis, we also proposed dimethyl sulfoxide as a novel coating strategy for DES.61 Dimethyl sulfoxide prevents vascular smooth muscle cell proliferation and migration, ie, the key mechanisms of restenosis; at the same time, dimethyl sulfoxide inhibits tissue factor upregulation in endothelial cells, vascular smooth muscle, and monocytes and prevents thrombotic occlusion in a mouse carotid injury model.61

Conclusions

The pathogenesis of stent thrombosis is still not fully understood. A combination of factors may be involved, including procedure-related factors, patient-related factors, and lesion
characteristics. With DES, biological properties such as thrombogenicity, i.e., induction of tissue factor, inhibition of reendothelialization of the stented segment, and distal endothelial dysfunction may increase the risk beyond that seen with BMS. Because premature cessation and resistance to antiplatelet drugs is associated with subacute and late DES thrombosis, prolonged dual-platelet inhibition must be considered, especially in high-risk patients, balanced against the risk of bleeding.

With nearly 6 million DES implanted, and in view of the high morbidity and mortality associated with it, stent thrombosis is an important healthcare issue that requires further clinical study. Consistent with this interpretation, the US Food and Drug Administration issued a statement acknowledging the importance of the increasing concern with respect to DES thrombosis. There was consensus that the on-label use of DES may be associated with a small increase in stent thrombosis compared with bare-metal stents, but that it does not lead to an increased risk of death or myocardial infarction, and that under these conditions, the benefits of DES outweigh a possibly increased risk for stent thrombosis; the panel left open the possibility, however, that off-label use of DES (i.e., use of DES outside the FDA-approved indications) may indeed be associated with an increased risk of death or myocardial infarction compared with the use of BMS owing to an increase in the rate of stent thrombosis.

Statements that second-generation DES appear to be less prone to stent thrombosis and thus “safer” than first-generation DES appear equally premature because the same uncertainty with respect to long-term safety prevails. Additional in vitro studies of compounds used on DES and autopsy studies of patients with DES dying of cardiac and noncardiac causes would help in understanding the mechanisms of stent thrombosis. Moreover, large-scale, non–corporate-sponsored registries and clinical trials are needed to reliably assess the “true” risk of stent thrombosis with DES. Control of data by non–corporate-sponsored data analysis centers under the auspices of the American Heart Association-American College of Cardiology-European Society of Cardiology, including cardiac surgeons, cardiologists, internists, and cardiac pathologists, is a prerequisite to the unbiased evaluation of current and future DES technologies.

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References
17. Pfisterer ME, Kaiser CA, Bader F, Brunner-La Rocca HP, Bonetti PO, Buser PT Late clinical events related to late stent thrombosis after stopping clopidogrel: prospective randomized comparison between drug-eluting versus bare-metal stenting. In: Program and abstracts from the American College of Cardiology 55th Annual Scientific Session; March 11–14, 2006; Atlanta, GA. Abstract 422-11.
20. Pfisterer ME, Kaiser CA, Bader F, Brunner-La Rocca HP, Bonetti PO, Buser PT Late clinical events related to late stent thrombosis after stopping clopidogrel: prospective randomized comparison between drug-eluting versus bare-metal stenting. In: Program and abstracts from the American College of Cardiology 55th Annual Scientific Session; March 11–14, 2006; Atlanta, GA. Abstract 422-11.


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