Vascular Responses to Drug Eluting Stents
Importance of Delayed Healing

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Abstract—Polymer-based sirolimus- (Cypher) and paclitaxel-eluting (Taxus) drug eluting stents have become the treatment of choice for patients with symptomatic coronary artery disease undergoing percutaneous coronary intervention (PCI). Although these stents reduce rates of restenosis compared with bare metal stents (BMS), late thrombosis, a life threatening complication, has emerged as a major safety concern. Our understanding of the pathophysiology of late DES thrombosis is derived from animal and human pathologic samples taken after implantation of these devices. These data indicate that both DES cause substantial impairment in arterial healing characterized by lack of complete reendothelialization and persistence of fibrin when compared with BMS. This delayed healing is the primary substrate underlying all cases of late DES thrombosis at autopsy. Several additional risk factors for late stent thrombosis such as penetration of necrotic core, malapposition, overlapping stent placement, excessive stent length, and bifurcation lesions represent additional barriers to healing and should be avoided if DES are to be used to minimize the risk of late thrombosis. Because the time course of complete healing with DES in man is unknown, the optimal duration of antiplatelet treatment remains to be determined. (Arterioscler Thromb Vasc Biol. 2007;27:1500-1510.)

Key Words: stents ■ thrombosis ■ endothelium

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olymer-based sirolimus (Cypher) and paclitaxel (Taxus) drug eluting stents (DES) have reduced rates of restenosis and target lesion revascularization (TLR) compared with bare metal stents (BMS) and launched a revolution in the interventional treatment of symptomatic coronary artery disease. However, enthusiasm for this technology has recently been dampened by concerns about late thrombosis (ie, stent thrombosis occurring more than 30 days after percutaneous coronary intervention), an event with often-catastrophic consequences.

Our understanding of the pathophysiology of late DES thrombosis is derived from the histological examination of animal and human arteries containing these devices. This review will focus on the cellular response that occurs when the drug and polymer contained in these devices interact with the vessel wall, especially as it relates to the process of late stent thrombosis.

Preclinical Evaluation of DES
Preclinical testing in both the porcine and rabbit models is an important part of the regulatory process used to determine the safety and efficacy of devices before human use. Comparative preclinical histological studies remain the most effective method of assessing the vascular responses to these devices before their introduction into humans. Although it is well-recognized that arterial repair after stent placement in animals occurs more rapidly than in man, the sequence of biological events associated with healing are remarkably similar. We will discuss below why the predictive value of the vascular healing responses for humans has for the most part not been shown.

Sirolimus and Paclitaxel and Their Effects on the Arterial Wall
Drug choice and release kinetics are probably the most important components of DES technology because they determine type of vascular response and time-course of healing. Both devices rely on the same overall strategy of using nontargeted drug delivery to the arterial wall using potent antiproliferative agents to prevent neointimal formation. However, because both sirolimus and paclitaxel also retard endothelial regrowth, there is the potential for impaired endothelial regeneration using this strategy. Moreover, recent data indicate that sirolimus may affect the growth and differentiation of endothelial progenitor cells, though the affect of locally delivered sirolimus on these cells and on their role in the process of reendothelialization remains unknown.

A considerable amount of data exists on how sirolimus and paclitaxel are different in terms of their effects on the arterial wall. Although both reduce restenosis by disrupting smooth
coronary arteries. Results of moderate-release Taxus paclitaxel eluting stents in the rabbit iliac and porcine muscle cells at both 28 and 180 days after implantation of macrophage infiltration, and an overall decrease in smooth formation is accompanied by persistent fibrin deposition, mitosis phase of the cell cycle.

Preclinical studies of sirolimus-eluting stents show a range of biological effects on arterial wall healing, inflammation, and neointimal growth. In a study by Klugherz et al little evidence of increased inflammation and delayed endothelialization was noted with 28-day sirolimus-eluting stents at both low and high doses used on the stents (64 or 196 μg per stent) compared with polymer-coated stents or BMS in rabbit iliac arteries. In another 28-day study, Suzuki et al reported higher amounts of accumulated fibrin with sirolimus-eluting stents (180 μg per stent) compared with BMS in porcine coronary arteries, although the degree of endothelialization was similar among groups. However, endothelialization of the luminal surface was assessed on a scale of 1 to 3 where 1 = 25%, 2 = 25% to 75%, and 3 = 75%; far too wide a range to draw any meaningful conclusions on the degree of endothelial coverage (BMS and Cypher both reported to be 2.9 ± 0.4 on this scale). Moreover, specific staining for endothelial cells or biologic markers of endothelialization was not performed.

Despite disparities in arterial wall pathology, both studies reported significant suppression of neointimal growth with sirolimus-eluting stents. More recently, Carter et al described the long-term effects of Cypher stents, again in porcine coronary arteries. Arterial inflammation characterized by giant cells gradually progressed from 90 to 180 days with a corresponding increase in neointimal formation. Granuloma formation was not mentioned. Although Western blot analysis of tissue at 90 days for PCNA and p27kip1 were similar among groups. However, endothelialization of the luminal surface was assessed on a scale of 1 to 3 where 1 = 25%, 2 = 25% to 75%, and 3 = 75%; far too wide a range to draw any meaningful conclusions on the degree of endothelial coverage (BMS and Cypher both reported to be 2.9 ± 0.4 on this scale). Moreover, specific staining for endothelial cells or biologic markers of endothelialization was not performed.

Paclitaxel is a cytotoxic drug known to suppress smooth muscle cell proliferation, their modes of action differ. Sirolimus inhibits the mammalian target of rapamycin (mTOR) and prevents the degradation of p27kip1, a cyclin-dependent kinase inhibitor that plays an important role in regulating vascular smooth muscle cell (VSMC) migration and proliferation. More pertinent to the process of arterial healing, sirolimus is also a potent inhibitor of endothelial cell proliferation by deactivating the p70 S6 kinase pathway, an essential step for cell cycle progression in response to growth factors.

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Paclitaxel is a cytotoxic drug known to suppress smooth muscle cell and endothelial cell proliferation and migration by disrupting microtubule dynamic, impacting cells in the mitosis phase of the cell cycle. Reduction in neointimal formation is accompanied by persistent fibrin deposition, macrophage infiltration, and an overall decrease in smooth muscle cells at both 28 and 180 days after implantation of paclitaxel eluting stents in the rabbit iliac and porcine coronary arteries. Results of moderate-release Taxus stents (1 μg/mm²) demonstrated a moderate inflammatory response without evidence of eosinophils and increased amounts of fibrin deposition with near complete endothelialization at 28 days. However, increasing the dose of paclitaxel resulted in a significant increase in luminal area contributed in part to medial wall necrosis, smooth muscle cell loss, and arterial dilation, thus emphasizing the narrow therapeutic index for this drug. Similar to sirolimus, paclitaxel also inhibits endothelial cell proliferation at nanomolar concentrations.

In contrast to these findings, other preclinical studies in the rabbit using systemic delivery of paclitaxel or everolimus did demonstrate delayed healing with significantly decreased endothelialization at 28 days by scanning electron microscopy (SEM) compared with placebo-treated animals. It was not until a study using commercially available Cypher and Taxus stents in the rabbit iliac artery model showed incomplete endothelialization as compared with matched BMS controls that the potential for these DES to produce incomplete endothelial coverage was realized, and a possible explanation for the late stent thrombosis occurred. In this study, incomplete reendothelialization was seen in both nonoverlapping and overlapping sites compared with BMS, though the differences were more pronounced in overlapping segments (Figure 1). Accompanying these findings were increases in fibrin and inflammatory cells, which persisted out to 90 days after stenting (supplemental Figure I, available online at http://atvb.ahajournals.org).

Before the introduction of DES, it had been shown that brachytherapy was associated with delayed arterial healing in preclinical studies. Brachytherapy intensively inhibits neointimal formation at early time points (ie, 28 days), whereas delayed arterial healing and “late catch up” had been documented in rabbit iliac artery model at 90 and 180 days.

Incomplete endothelial coverage and persistent fibrin deposition were demonstrated even at later time points. Clinically, brachytherapy was also associated with late stent thrombosis, which was documented in both clinical trials and in autopsy studies. The brachytherapy paradigm reinforces the importance of preclinical studies in predicting human responses.

Shortcomings of Existing Models

A major criticism of earlier preclinical studies leading to the FDA approval of both Cypher and Taxus stents is their failure to detect significant differences in the healing responses of the arterial wall when compared with BMS at 28 days when human angioscopic and autopsy data clearly demonstrate significant differences in healing. An important explanation may be that the 28 day time point chosen for examination may not be appropriate for the assessment of endothelium in the pig model. Using SEM, we established the temporal course of healing in both the porcine and rabbit models by comparing nonoverlapping and overlapping sites at 28 days. Using SEM, we established the temporal course of healing in both the porcine and rabbit models by comparing nonoverlapping and overlapping sites at 28 days. Using SEM, we established the temporal course of healing in both the porcine and rabbit models by comparing nonoverlapping and overlapping sites at 28 days. Using SEM, we established the temporal course of healing in both the porcine and rabbit models by comparing nonoverlapping and overlapping sites at 28 days.

Another aspect that deserves mention is the importance of atherosclerosis and other plaque-related factors such as thrombus in affecting the arterial responses to these devices.
To date, preclinical testing has relied on normal animal models to determine drug levels, device efficacy, and safety. Lipid as well as thrombus within the stented atherosclerotic plaque may influence drug distribution and retention.\textsuperscript{25,26} Given the hydrophobicity of both sirolimus and paclitaxel, it is unclear whether atherosclerosis prolongs tissue retention leading to long-term biological effects. The importance of this issue is underscored by the striking disparity between relatively short time period of drug release kinetics and tissue level in animals and the very long term delay in healing (ie, \(>50\) months) seen in humans at autopsy.\textsuperscript{24} Our own data in the atherosclerotic rabbit iliac model indicates that at least in the case of the Taxus stent, this model delays reendothelialization with only 64\% endothelial coverage at 42 days after deployment of a bare metal stent (BMS) and drug-eluting stent implanted in the rabbit iliac artery model for 28- and 90-days. Note significantly less endothelialization in Cypher and Taxus DES as compared with bare metal Bx Velocity and Express, especially at overlapping sites at 28 days. At 90 days the luminal surface in overlapping DESs is still not fully endothelialized. Arrows indicate the overlapping regions.

**Figure 1.** Scanning electron micrographs from overlapping bare metal stents (BMS) and drug-eluting stent implanted in the rabbit iliac artery model for 28- and 90-days. Note significantly less endothelialization in Cypher and Taxus DES as compared with bare metal Bx Velocity and Express, especially at overlapping sites at 28 days. At 90 days the luminal surface in overlapping DESs is still not fully endothelialized. Arrows indicate the overlapping regions.

**Figure 2.** Comparison of endothelialization by scanning electron microscopy (SEM) in pig coronary and rabbit iliac arteries after deployment of a bare metal Vision stent. Both pig and rabbit arteries were examined at serial time points after stent deployment. Endothelial coverage was complete in the pig by 14 days whereas in the rabbit it was complete by 21 days (A and B). In C the graph depicts the percentage of endothelial coverage in the pig and rabbit as a function of time. Note the slower pace of endothelialization in the rabbit vs pig.
single stent deployment as compared with near complete coverage in a normal rabbit model at 28 days.\textsuperscript{18,27} The importance of using more complex models that more closely mimic the complexities of human coronary disease is reinforced by recent data demonstrating increased risk of thrombotic events for off label (eg, treatment of myocardial infarction, bifurcations, etc.) rather than on label DES use.\textsuperscript{28}

**Inflammatory Responses**

In addition to plaque related factors, significant inflammatory responses to these devices also may occur in animals and in humans. In contrast to BMS, both polymeric stents provoke eosinophilic/heterophilic infiltration of the arterial wall in the rabbit (which are more impressive in overlapping segments). The pig also shows progressive granulomatous and eosinophilic reaction to Cypher stent starting at 28 days and increasing out to 6 months (1 month, 14%; 3 months, 43%; 6 months, 60%; R.V., unpublished data, 2006; Figure 3). The likely explanation for these findings is a local hypersensitivity reaction to the nonerodable polymers used in both stents (Cypher-polyethylene-co-vinyl acetate [PEVA] and poly n-butyl methacrylate [PBMA], Taxus-SIBBS [poly(styrene-b-isobutylene-b-styrene)], although it is still possible the drug may also be a culprit. However, in pig model, the hypersensitivity reaction peaks only after the complete release of drug (ie, >60 days), reinforcing the notion that polymer may be the cause. These reactions mimic the local hypersensitivity reaction, which has been reported in a small number of patients mostly receiving the Cypher stents (discussed below), albeit to a much lesser degree.\textsuperscript{24}

**Endothelial Dysfunction**

In addition to the examination of endothelial regrowth of the stented segment, understanding the impact of DES on endothelial function is paramount because recent clinical reports suggest that in addition to delayed healing, DES may also impair endothelial responses to acetylcholine and exercise mediated vasodilation in humans.\textsuperscript{29,30} The ability of an intravascular device to facilitate functional endothelial regrowth is crucial because these cells provide essential antithrombotic factors in addition to structural integrity to maintain stent patency. Although scanning electron microscopy (SEM) is a useful tool for morphological evaluation of endothelial coverage over stent surfaces because it allows for en face visualization, it lacks the ability to gauge the expression and precise location of candidate markers of endothelial function.

Recent in vitro studies examining the effects of paclitaxel and sirolimus on endothelial cells demonstrate increase in tissue factor mRNA and protein expression.\textsuperscript{31,32} Another report suggests that these agents also increase local production of plasminogen activator inhibitor (PAI)-1 in cultured
endothelial cells. These findings suggest that the late thrombotic risk of DES may be a function of both the extent of endothelial coverage as well as the ability of these cells to conduct their normal functions. To date, no preclinical studies have examined markers of endothelial function after DES placement. We have begun performing immunohistochemical staining for markers of endothelial anticoagulant function such as thrombomodulin and PECAM-1 using confocal microscopy on DES implanted in the rabbit iliac model (R.V., unpublished data, 2006).

Although endothelial dysfunction remains a theoretical concern, pathologic findings from patient dying of late stent thrombosis demonstrate lack of endothelialization with superimposed thrombosis. We have not seen any cases of late stent thrombosis in the setting of complete endothelialization.

Clinical and Pathologic Data

The approval of the current generation polymeric Cypher and Taxus DES by the FDA was based on short-term (<1 year) randomized clinical trial data demonstrating impressive reductions in restenosis compared with the standard bare metal stents without increases in serious adverse cardiac events. However, these studies were not powered to examine safety endpoints such as late thrombosis. Moreover, the exclusion of factors known to increase complication rates such as myocardial infarction and ostial, bifurcation, or severely calcified lesions minimized the chances that any increased thrombotic risk would be seen. These “off label” uses comprise the majority (60%) use for DES today.

A number of clinical case reports first raised concerns about an increased risk of late stent thrombosis after DES implantation with some of these cases occurring more than 1 year out, a very different temporal pattern of risk than that seen for BMS. Clinical data revealed certain patient related risk factors of late stent thrombosis such as diabettes, renal failure, low ejection fraction, and withdrawal of antiplatelet therapy, with the latter being the most powerful predictor. A number of presentations at the European Heart Association meeting in Barcelona, Spain in 2006 such as the BASKET-LATE (Basil Stent Cost-Effectiveness Trial – Late Thrombotic Events) trial demonstrated higher rate of cardiac death and nonfatal MI in DES versus BMS after stoppage of clopidogrel at 6 months with 18 month follow-up (4.9% versus 1.3%; \( P=0.01 \)). This study was the first randomized trial to confirm the excess thrombotic risks associated with DES. Recently, 4 meta-analyses and 1 registry study from Sweden have been reported on the subject of DES safety. Although these data provide conflicting results especially regarding the safety of on-label use, most experts agree there is an increase risk of late thrombosis and perhaps mortality resulting from off label use.

Our understanding of the pathophysiology of late DES thrombosis is derived from human pathologic samples taken from patients who received these devices. Because of the small number of samples available (compared the millions of these devices implanted), the issue of selection bias is often raised as an inherent limiting factor in whether these findings apply to the majority of patients who receive DES and survive. It is true that a large number of patients in our database received DES for non-FDA approved indications and so may not reflect the arterial pathologic changes that occur when these stents are placed only for approved uses. However, because a preponderance of patients receive DES for these same non-FDA approved indications, we believe it is likely that our pathologic results are translatable to most patients receiving DES.

Detailed morphometric and histological analysis of these specimens have allowed us to understand how the vascular responses and healing characteristics of the current generation of Cypher and Taxus DES differ from that of BMS. In addition, we have also learned about specific pathologic risk factors for late DES thrombosis.

We compared 23 autopsies of human DES implants of more than 30 days duration to 25 bare metal stent implants matched for age, sex, stented artery and duration of implant, and demonstrated delayed arterial healing, as evidenced by persistence of fibrin, minimal neointimal thickening, and incomplete endothelialization compared with BMS (Figure 4). Poor endothelial cell coverage of the lumen was consistently documented in DES cases regardless of the duration of implantation, which contrasted with the pathologic findings from patent BMS showing abundant neointimal smooth muscle cells and proteoglycan deposition with minimal persistence of fibrin deposition (Figure 5A). Endothelialization was complete in most BMS sections consistent with earlier pathologic studies which have suggested near complete endothelialization by 3 to 4 months with α-actin positive smooth muscle cell presence peaking at 9 to 18 months. These findings are complimented by angioscopic studies in humans receiving polymer based sirolimus eluting stents or BMS. Kotani reported that 13 of 15 sirolimus stents had incomplete neointimal coverage a mean of 3 to 6 months after stent placement, whereas all 22 BMS demonstrated complete coverage.

Late stent thrombosis, defined as any platelet rich thrombus occupying >25% of lumen >30 days after DES implantation, was observed in 14 of 23 patients receiving DES. The major pathologic finding distinguishing late thrombosed from patent DES was evidence of a significantly greater delay in arterial healing characterized by lack of endothelialization and persistent fibrin deposition at a mean of approximately 6 months after DES implantation (Figure 5B). We also reported several procedural and pathologic risk factors for late stent thrombosis such as ostial or bifurcation stenting, malposition/incomplete apposition of struts, and strut penetration into a necrotic core. Many of these underlying lesions were also reported as risk factors for late bare metal stent thrombosis and represent additional barriers to healing. However the timing of late stent thrombosis in BMS was significantly earlier than those in DES (BMS; median, 70 days, interquartile range; 33 to 127, versus DES; median, 173 days, interquartile range; 66 to 433, \( P=0.04 \)).

Underlying plaque morphology may affect the rate of healing when stent struts penetrate deeply into the necrotic core and are not in contact with cellular areas. Lipophilic agents such as sirolimus and paclitaxel are likely to persist longer when the struts are located in the necrotic core as this area is avascular. Arterial branch points may also predispose
toward thrombosis by inducing flow disturbances and changes in shear stress.\textsuperscript{47,48} Heavily calcified plaques may prevent uniform strut deployment leading to malapposition and flow disturbances that could influence the pattern of arterial healing through shear stress induced growth stimuli.\textsuperscript{49}

Late-acquired incomplete apposition is reported in 8.7% and 8.4% of patients after Cypher and Taxus stent placement, respectively, but has not been linked to an increase risk of late stent thrombosis.\textsuperscript{50} Without baseline and follow-up intravascular ultrasound (IVUS) data, our own autopsy data are limited by the fact that it is difficult to determine whether incomplete apposition is acquired or the result of incomplete stent expansion during the index procedure. Nonetheless, it appears by pathology that lack of strut apposition to the vessel wall retards arterial healing as these struts remaining uncovered. Also, late malapposition pathologically is associated with presence of thrombus between stent strut and the underlying plaque. We found a higher prevalence of incomplete struts apposition to the vessel wall in lesions with thrombosis compared with those without (29% of lesions versus 6%, $P=0.02$; R.V., unpublished data, 2006). Kimura et al investigated 31 patients with acute incomplete apposition and found that 75% of those sites of incomplete apposition were persistent at follow-up. Although none of them suffered stent thrombosis during the follow-up period, the long term effect of incomplete apposition cannot be determined by such a small number of patients and/or a short term follow-up.

In addition to these procedural and pathologic risk factors, both sirolimus and paclitaxel also contribute indirectly toward creating a prothrombogenic and proinflammatory environment.\textsuperscript{51} Sirolimus inhibition of mTOR increases both thrombin and tumor necrosis factor (TNF)-$\alpha$ expression in endothelial cells and monocytes at concentrations consistent with the current stent-based dose.\textsuperscript{32,52,53} Paclitaxel activates c-Jun NH$_2$-terminal kinase, an important signaling molecule controlling endothelial and monocyte tissue factor expression at similar stent-based concentrations.\textsuperscript{51}

The increasing use of DES for a wide variety of complex clinical and anatomic situations such as these that have not been evaluated in randomized studies was an inevitable consequence given the enthusiasm behind DES introduction. The increased risk for death and thrombosis demonstrated for “off label” use is understandable in light of the healing characteristics of these devices especially when placed in anatomic or lesions consideration that provide additional barriers to healing.

For the present, the use of DES for lesions or anatomic considerations such as penetration of necrotic core (as visualized by IVUS), bifurcation stenting, excessive stent length, overlapping stents should be avoided. For instance, penetration of necrotic core is frequently documented at autopsy in patients with acute myocardial infarction (AMI). Although
two pivotal studies showed similar rates of stent thrombosis in AMI patients treated with BMS and DES, the limited duration of follow-up (ie, 1 year) precludes any definitive conclusion about the safety of this practice.54,55 Our own observational pathologic studies show a tendency for lesions with penetration of necrotic core by stent struts to be associated with late stent thrombosis (unpublished data, 2006).

Another cause of late thrombosis involves a massive local hypersensitivity reaction likely provoked by the respective polymers employed in each type of DES. One case involved the occurrence of late in-stent thrombosis secondary to a hypersensitivity reaction at 18 months after Cypher stent implantation.56 The morphological changes were localized to the area of the stent and consisted predominantly of CD45-positive lymphocytes and eosinophils (Figure 6). However, the causal relationship between massive local polymer induced-inflammation and late stent thrombosis has only been proven in a minority of patients with late DES thrombosis. To date, in our database of 105 DES, we have observed hypersensitivity reactions in 5 cases, 4 with Cypher and 1 with Taxus. When hypersensitivity occurs in Cypher stents, the inflammatory cells are pervasive throughout the whole stent, whereas in the 1 case involving a Taxus stent, only focal eosinophilic infiltrate and some lymphocytes around occasional stent struts were seen. The majority of cases of late thrombosis do not show massive local eosinophilic infiltration and overall DES stented arteries have inflammation scores no different from that of BMS, with the exception that local eosinophils, (which are significantly greater in the DES than in BMS) appear to be associated predominantly with the Cypher stent (R.V., unpublished data, 2006).24

More recently, based on a larger number of autopsy cases, we reported the specific morphometric and histological parameters that significantly correlate with late thrombosis.46 Multiple logistic generalized estimating equations (GEE) modeling demonstrated that endothelialization was the best predictor of thrombosis. The morphometric parameter that
best correlated with endothelialization was the ratio of uncovered to total stent struts per section (RUTSS), suggesting that neointimal coverage of struts could be used as a surrogate for endothelialization (supplemental Figure II). A univariable logistic GEE model of occurrence of thrombus in a stented section versus RUTSS demonstrated a marked increase in risk for LST as the number of uncovered struts increased.

There was also a significant correlation between stent struts lacking neointimal coverage and the number of struts surrounded by platelet-rich thrombi. We found heterogeneity of coverage of stent struts, both within individual cross sections as well as between sections from the same stent. Within the same stent, whereas some struts showed healing as demonstrated by neointimal growth, others remain bare and served as a nidus for mural thrombus formation. The middle section of the stent (versus the proximal and distal ends) was the most common location of stent struts lacking neointimal coverage, and this was also the most common site of thrombus formation.

Intuitively, the lack of neointimal coverage in the mid portion of the stent could be assumed effect of greater concentration of drug in this region. Stent design issues undoubtedly influence target drug-delivery as strut thickness, interstrut spacing, and strut apposition to the arterial wall likely play an integral role. This together with complex systems of elution and differing drug-tissue affinities could further complicate these relationships. Moreover, hemodynamic effects created principally by diffusion constants and areas of blood stagnation (eddies) principally caused by luminal protrusion of struts might lead to drug pooling allowing uneven drug distribution in the arterial wall.

Refined computational methods with adjustments for strut position, shape, and coatings have advanced the field by allowing us to model the effects of these variables on local drug delivery. Collectively, these mathematical simulations have raised awareness that elution of drug is not simply a property of drug diffusion and washout as previously thought but support the argument that flow (which is influenced by parameters such as strut size), interstrut distances, number of struts, overlapping struts all determine the amount of drug deposition and distribution beyond levels achieved exclusively by arterial wall contact. In addition, similar modeling confirms the importance of strut position on spatial concentration since struts penetrating into the arterial wall confer the most optimal drug distribution compared with half or non-embedded stent struts.

Despite the relationships defined by computational models, the understanding of fluid dynamics on drug-tissue interaction becomes increasingly challenging in real-world scenarios because stent struts generally do not evenly contact the arterial wall, as varying degrees of strut penetration is dictated by lesion composition ranging from heavily calcified to fibrotic tissue to lipid-rich plaques. These underlying plaque characteristics also determine the stent strut spacing (and therefore drug distribution) and may ultimately help explain heterogeneity of healing within DES. Our own human pathologic data demonstrate a good correlation between the mean number of uncovered struts per section and the average distance between stent struts \((r=-0.41, P=0.001)\), with the majority of uncovered stent struts showing less interstrut distance than covered stent struts.

Our data raise the possibility that a more liberal approach to reductions in late loss and suppression of neointimal

Figure 6. Histological findings from a 58-year-old male who died of late stent thrombosis. Two Cypher stents had been placed in the left circumflex artery for unstable angina 18 months before presentation with chest pain. A histological examination showed focal malapposition with aneurysmal dilatation (double arrow) and stent malapposition with occlusive thrombus in the lumen. B. The vessel wall is highly inflamed and high power images show an extensive inflammatory infiltrate consisting mostly of eosinophils (C and D). E shows Luna stain for eosinophils, F T-lymphocytes (CD45Ro), and G macrophages (CD 68). (Reproduced with permission from Virmani et al.)
growth may be able to reduce the risk of thrombosis by encouraging a higher percentage of strut-related neointimal coverage. This relationship has so far not been seen for the current generation FDA-approved DES. Although rates of late loss for Cypher are approximately 0.2 mm lower than for Taxus, the risks of late thrombosis are similar according to published studies, though it must be acknowledged that every clinical trial has been underpowered to detect differences in thrombosis rates. One reason for this discrepancy may be the distinctive arterial reaction produced by each type of stent. Our pathologic data demonstrate that Taxus stents produce more fibrin deposition than Cypher stents in humans (fibrin score 2.3±1.4 versus 1.6±1.3, P=0.08; R.V., unpublished data), and it is perhaps this increased fibrin that predisposes toward thrombosis despite the fact that Taxus stents have more neointima. Also, uneven polymer and drug distribution within the stent could give rise to uneven coverage of strut struts. In addition to delaying reendothelialization, the choice of drug and its particular properties may have important influences on the local response to stenting. It remains to be determined whether newer DES such as Endeavor with a reported late loss of 0.6 mm will demonstrate a lower late thrombosis risk.

The delay in arterial repair seen after DES implantation means prolonged antiplatelet therapy is needed to minimize (but not obviate) the risk of thrombotic complications. This has been confirmed in clinical studies which have demonstrated that the most important risk factor for late DES thrombosis is withdrawal of antiplatelet therapy. It is not surprising that these poorly healed sites pose a significant risk for complete thrombosis in the absence of adequate antiplatelet blockade. Although the American Heart Association and the American College of Cardiology (ACC) recommend 12 months of dual antiplatelet therapy with aspirin and clopidogrel, recent data from the SIRTAx and Post-SIRTAx registries in Bern and the RESEARCH and T-SEARCH registries in Rotterdam indicate that stent thromboses continue to occur steadily, at a constant rate of 0.6% per year at least out to 3 years after stent implantation and perhaps beyond. In the overall group (n=8146), the 3-year percentage cumulative all-cause mortality was 10.3%. It remains uncertain whether reendothelialization with DES is delayed or remains persistently incomplete, and this has important implications for the duration of risk for patients receiving these devices. In either case, the optimal duration of antiplatelet therapy remains unknown.

It must also be acknowledged that it is not entirely clear that being on dual antiplatelet therapy is completely protective against late stent thrombosis. For instance, seven of 14 subjects in our own study died of late DES thrombosis while on dual antiplatelet therapy. Moreover, in the group dying with patent DES in place, mean endothelialization was only 66.1% at an average of 181 days after stent placement. In terms of population attributable risk, Tsai et al estimated that only 15% to 30% of stent thromboses are attributable to clopidogrel discontinuation. These pathologic and clinical findings highlight the complexity of late stent thrombosis and suggest that other mechanisms including local plaque/anatomic and patient considerations may be operative. These findings also call into question the assertion that extending the duration of antiplatelet therapy will ameliorate the risk of late stent thrombosis.

Beyond Late Loss: Measures of DES Safety

The primary purpose of DES technology is to reduce the incidence of clinical restenosis after coronary stenting. However, the degree of neointimal suppression should not be the only measure used to evaluate new devices. The goal of DES technology should be to balance the benefits of restenosis prevention against the risks of delayed healing and late stent thrombosis. The currently used angiographic end points for DES trials such as late loss and binary restenosis are inadequate to determine whether stent surfaces are completely healed. Moreover, currently available imaging modalities such as IVUS and optical coherence tomography (OCT) are incapable of evaluating endothelialization of stent surfaces in man because their limited resolution cannot distinguish fibrin deposition from smooth muscle cell and endothelial cells. Therefore, until better clinical tests become available, we are dependent on autopsy and animal studies to evaluate how well new devices endothelialize.

Our understanding of the healing characteristics of newer devices would also grow if we made pathologic examination a mandatory part of the evaluation of subjects who die while enrolled in randomized trials of these devices. Even in cases of death that are not stent-related, examination of the stented segment can help us learn more about the local cellular/healing responses to these devices. We have relied on autopsy data to understanding the drawbacks of the current generation DES and we are already understanding how to design better drug eluting stents based on this data.

We have learned that DES performance should not be based solely on the degree of neointimal suppression but rather on the cellular response to these devices especially as it relates to arterial healing. We need to strike a more even balance between these two ambitious efforts. In the short-term this probably means creating devices which have greater late loss (and therefore encourage more neointimal strut-related coverage). In the long-term this may mean smooth muscle specific targeting with antiproliferative agents that do not affect endothelial regeneration but result in neointimal suppression.

In the coming year, it is expected the FDA will approve 3 new DES, each containing some advance either in the bare metal stent backbone, better polymer (which is degradable in some cases), or in the total loaded dose of drug.

Conclusions

The strength of the current polymer based sirolimus (Cypher) and paclitaxel (Taxus) DES lies in their ability to prevent restenosis in a wide variety of patients. However, both cause significant delay in arterial healing manifested by persistent fibrin deposition and poor endothelialization when compared with sites of bare metal stent implantation. These observations offer a pathophysiologic explanation for the phenomenon of late thrombosis seen in clinical trials and registries of DES and underscore the need for continued antiplatelet therapy in patients receiving these stents. Short of large
randomized trials powered to detect significant differences in thrombosis, preclinical evaluation of DES may be the most feasible method to distinguish the healing characteristics of new DES. Animal and human pathologic studies have played an important role in changing the way we view this technology in its current state and directing future developments.

Disclosures


References


Vascular Responses to Drug Eluting Stents: Importance of Delayed Healing
Aloke V. Finn, Gaku Nakazawa, Michael Joner, Frank D. Kolodgie, Erik K. Mont, Herman K. Gold and Renu Virmani

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Ratio of uncovered to total stent struts/section against Endothelialization (%)

$r^2 = 0.87$

$P < 0.0001$