The present and future of drug-eluting stents

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The only widely accepted way to reduce restenosis rate after percutaneous balloon angioplasty has been the use of coronary bare metal stents, and the last decade has witnessed a prompt and widespread adoption of bare metal stents that has revolutionized the field of interventional cardiology. The new millennium has seen the recent development of drug-eluting stents (DES), allowing controlled release of a drug directly to the injured artery, which seem to have prevented by large the problem of in-stent restenosis.

The goal of this review was to summarize recent laboratory and clinical investigations concerning the effects of DES in various settings relevant to coronary heart disease. In the experimental setting, we examine the intracellular signaling and the role of smooth muscle cells after vascular injury. We also discuss recent observations from our laboratory showing the effects of coating *per se* on cell apoptosis and proliferation. In the clinical setting, the effects of DES in patients with stable or unstable angina pectoris is examined in detail for the relevant implications both in the treatment and prognosis. The results of a meta-analysis on the effects that have been overlooked in individual studies are reported which show a striking reduction in bypass surgery after DES implantation.

Finally, we discuss the potential role of new materials and technologies (i.e., nanotechnology) that will improve DES performance allowing other future clinical applications in patients with ST-elevation myocardial infarction, vulnerable plaques, insulin-dependent diabetes mellitus, etc.

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Introduction

Restenosis, which is defined as "the arterial healing response after injury incurred during transluminal coronary revascularization", has been the principal drawback of percutaneous coronary interventions (PCI) since their introduction nearly 25 years ago[1].

The only widely accepted way to reduce restenosis rate has been the use of coronary bare metal stents (BMS), and the last decade has witnessed a prompt and widespread adoption of BMS that has revolutionized the field of interventional cardiology[2,3]. Although the use of coronary BMS is associated with lower rates of restenosis than balloon angioplasty alone, rates of up to 40% have been reported in some series, and treatment options of in-stent restenosis are often unsatisfactory, with high recurrence rates after further intervention. Neointimal hyperplasia begins soon after intervention proportionally to the degree of injury[4] and is the mechanism responsible for restenosis after stenting[5].

Previous pharmacological trials on systemic drugs to prevent neointimal hyperplasia have failed to prevent restenosis partially because effective local concentrations of the drug were not achievable without producing at the same time systemic toxicity[6].

A novel solution to this problem has been the recent development of drug-eluting stents (DES), allowing controlled release of a drug directly to the injured artery[2,3]. Numerous pharmacological agents for local delivery have been tested for their potential to inhibit restenosis. However, two DES with sirolimus and paclitaxel received recently Food and Drug Administration approval for clinical use.

Since the introduction of DES, a number of randomized trials have compared BMS and DES for the treatment of patients with coronary artery disease[2,3]. These trials demonstrated striking preliminary results that have generated enormous expectations and clinical demand for DES. However, to date many of the completed trials have been of small sample size, with clinical outcome as secondary endpoint, and due to the limited number of side effects (stent thrombosis, myocardial infarction and death) in both arms, they could not be con-

clusive in terms of potential side effects of these innovative devices[3]. Moreover, long-term clinical outcomes are now available for only two of these trials.

On these premises, the goal of this review was to summarize the basic scientific background from where DES developed and to rigorously and critically analyze the available clinical data on the efficacy of DES, trying to highlight any missing information and the possible limitations. Finally, we will formulate some of the future directions for further improvement in DES technology.

The experimental background of drug-eluting stents

Mechanisms of restenosis after balloon angioplasty and stenting. Restenosis is considered a local vascular manifestation of the general biologic response to injury[3]. Post-balloon angioplasty restenosis is thought to involve primarily negative remodeling and, partially, neointima hyperplasia[7-9]. Histologically, however, in-stent restenosis is quite distinct from restenosis after balloon angioplasty[7]. In fact, intravascular ultrasound studies suggest that coronary stents provide mechanical scaffolding that virtually eliminates long-term negative remodeling and that in-stent restenosis is largely a result of vascular smooth muscle cell (VSMC) proliferation, which is exaggerated after stent deployment due to the high-pressure technique of stent deployment[4,5] (Fig. 1).

A commonly accepted model of the response to arterial injury suggests that growth factors are released after injury, thereby changing the composition of the extracellular matrix and triggering a proliferation and migration program. VSMC undergo a phenotypic modulation from a contractile to a synthetic phenotype (dedifferentiation), proliferate into the media, migrate from the media into the intima, and subsequently form the neointima (Fig. 1). Moreover, it has been shown that circulating progenitors give rise to the part of VSMC that contribute to arterial remodeling after vascular injury[3]. These data, however, have been challenged by recent findings that were unable to confirm the invasion of bone marrow-derived cells into the intima of atherosclerotic vein grafts[3]. Nevertheless, wherever they originate, VSMC that form the neointima tissue remain the main target if restenosis is to be challenged.

Vascular smooth muscle cell intracellular signaling after vascular injury. In the past few years, many investigators have focused their attention on the relative importance of single receptors in the complex mechanism of VSMC growth control after vascular injury[10]. However, it is unlikely that the inhibition of only a single receptor would be clinically relevant to the prevention of in-stent restenosis. Therefore, in the last few years, we have studied mainly the common pathways that multiple receptors employ to transmit mitogenic signals from the membrane to the nucleus in VSMC after arterial injury; in particular, the role of the *ras-raf*-mitogen-activated protein kinase (MAPK) pathway and the cyclic adenosine monophosphate (cAMP)-dependent signaling in activated VSMC[10-16] (Fig. 2).

Ras proteins are key transducers of mitogenic signals from the plasma membrane to the nucleus in many cell types. Several mutants of *ras* (such as N17 H*ras* and L61,S186 H*ras*) act in a dominant negative manner to block normal *ras* activation[11]. N17 H*ras* has reduced affinity for guanosine triphosphate (GTP) and is defective in the final step of the exchange process, displacement of guanine-exchange factor by GTP, and sequestering of guanine-exchange factor into dead-end complexes[11]. This mutant has been used to remove guanine-exchange factor activity from cells so that activation of endogenous *ras* proteins cannot occur. We investigated

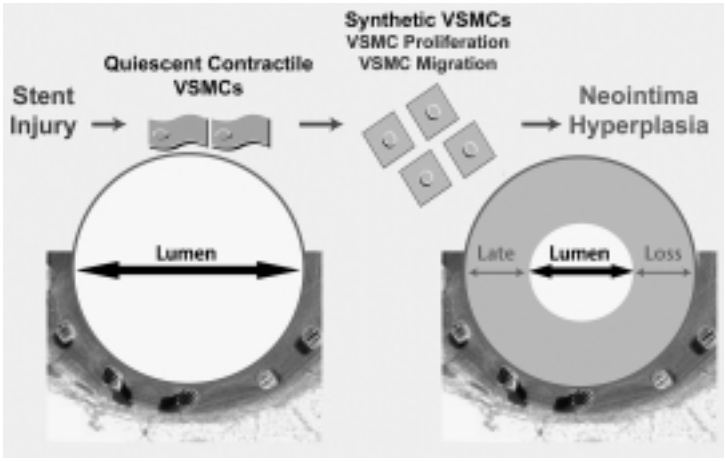

Figure 1. *The pathophysiology of in-stent restenosis. Late luminal loss after stenting reflects vascular smooth muscle cell (VSMC) proliferation after the injury induced by stent deployment. Upon the injury, VSMC undergo a phenotypic modulation from a contractile to a synthetic phenotype (dedifferentiation), proliferate into the media, migrate from the media into the intima, and subsequently form the neointima.*

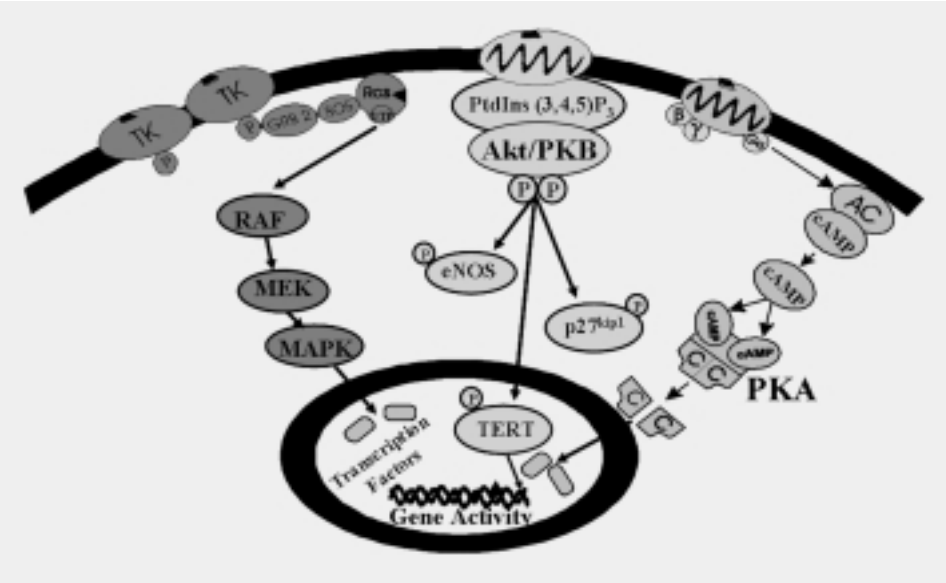

Figure 2. *Schematic diagram of the main intracellular pathways regulating vascular smooth muscle cell proliferation. For brevity three main signaling pathways from the membrane to the nucleus are discussed. Once activated by the binding with specific growth factor, the tyrosine kinase (TK) receptor triggers a cascade of tyrosine phosphorylations, which lead to the formation of a ternary complex connecting the receptor to ras via adaptor molecules (GRB-2 and SOS). The net result of this chain of events is the activation of ras by guanosine triphosphate (GTP) binding. Active ras binds the serine protein kinase raf that activates MEKK (mitogen-activated protein kinase - MAPK). This cascade culminates in the activation of the nuclear transcription factors. On the other side of the figure the cyclic adenosine monophosphate (cAMP) pathway is shown. The prototypic receptor containing a 7 transmembrane segment, upon binding the hormone, activates the heterotrimeric G protein cascade. Gαs (GTP-bound) activates adenyl cyclase (AC), which increases cAMP production. cAMP binds the tetrameric enzyme protein kinase A (PKA) and dissociates the enzyme by releasing active catalytic subunit (C). A fraction of C migrates to the nucleus and phosphorylates the transcription factors which stimulate the transcription of several cAMP-responsive genes, leading to the suppression or stimulation of cellular growth. Finally, in the center of the picture PI3K-Akt pathway is shown. Ligation of receptors leads to the recruitment of members of the PI3K family of lipid kinases to the plasma membrane where they encounter the substrate lipid phosphatidylinositol 4,5 bisphosphate, leading to formation of 3,4,5 PIP3. This molecule recruits Akt/PKB that becomes activated. Cytosolic substrates of Akt include p27^{Kip1} and endothelial nitric oxide synthase (eNOS), whereas nuclear substrates include TERT (the catalytic subunit of the telomerase complex).*

the role of *ras* proteins in the vascular response to arterial injury *in vivo* by inactivating cellular *ras* function in rats in which the common carotid artery was subjected to balloon injury. In animals treated with N17 H-*ras*, a significant reduction in neointima formation (-55%) was observed 14 days after balloon injury compared with control animals[11]. Interestingly, simvastatin prevents smooth muscle cell proliferation *in vitro* and neointimal formation *in vivo* after experimental balloon angioplasty[17], mainly through inhibition of *ras* activation[17,18].

cAMP is involved in the regulation of a variety of cellular functions, such as cell proliferation and differentiation. To evaluate the effects of cAMP on VSMC proliferation, we treated cultures of these cells *in vitro* with different concentrations of 8-Br-cAMP and analyzed cell growth at different treatment periods[12]. cAMP markedly inhibited VSMC proliferation. We next sought to determine whether cAMP was able to inhibit VSMC proliferation *in vivo* by balloon-injuring rat carotid arteries. At the time of balloon injury, we locally delivered 8-Br-cAMP. In the 8-Br-cAMP group, a significant reduction in neointima (-54%) was observed 14 days after vascular injury compared with the control group[12]. An inhibition of neointimal formation after vascular injury was also observed in other experiments[14], in which systemic administration of 8-Cl-cAMP reduced protein kinase A (PKA) RIα subunit expression and upregulated PKA RIIβ-subunit expression. Notably, we demonstrated that A-kinase anchor protein (AKAP), downstream molecules of the cAMP-PKA cascade, inhibited VSMC proliferation *in vivo* by increasing p27^{Kip1} protein levels[15].

The drugs: sirolimus and paclitaxel. The ideal antirestenotic drug for local delivery should have potent antiproliferative effects but yet preserve vascular healing, i.e. re-endothelialization. In addition, the drug should not incite thrombosis or inflammation[19]. Anticancer and anti-transplant rejection agents are now being considered in the fight against restenosis. Only a few agents have demonstrated clinical efficacy, but the search for is still ongoing. Drugs that interfere earlier in the cell cycle (G1 phase) are generally considered cytostatic and potentially elicit less cellular necrosis and inflammation compared with agents that affect the cell cycle in a later stage (beyond the S phase)[19]. Here we will shortly summarize the two drugs of the only two Food and Drug Administration approved DES: sirolimus and paclitaxel.

Sirolimus is a macrolide antibiotic with potent antifungal, immunosuppressive, and antimitotic properties[19]. The drug is produced by cultured *Streptomyces*

hyroscopicus. Rapamune was approved by the US Food and Drug Administration for the prophylaxis of renal transplant rejection in 1999. Shortly after this approval, the first sirolimus-eluting stents (SES) were implanted in human coronary arteries.

Paclitaxel is a microtubule-stabilizing agent with potent antitumor activity[20]. Unlike other antimitotic agents, paclitaxel shifts the cytoskeleton equilibrium toward assembly, leading to reduced vascular cell proliferation, migration, and signal transduction. Paclitaxel is highly lipophilic, resulting in a rapid cellular uptake and a long-lasting effect in the cell[20].

The present of clinical use of drug-eluting stents

The sirolimus-eluting stents. The first small randomized study on SES, the Randomized Study with the Sirolimus-Coated Bx Velocity Balloon-Expandable Stent in the Treatment of Patients with de Novo Native Coronary Artery Lesions (RAVEL)[21], showed an impressive reduction in the frequency of in-stent restenosis with SES (from 26.6 to 0%).

In the more recent 1058-patient SIRIUS largest trial[22] the use of SES reduced the rate of target vessel failure from 21% with a standard stent to 8.6% with a SES ($p < 0.001$). The frequency of the need for target lesion revascularization was also significantly reduced (16.6% in the standard-stent group vs 4.1% in the sirolimus-stent group, $p < 0.0001$) (Fig. 3). Patients with diabetes composed 26% of the study population in this trial, and the rate of restenosis was considerably higher in this subgroup (about 18% in the sirolimus-stent group vs about 51% in the standard-stent group)[22].

The E-SIRIUS trial enrolled 352 patients in whom one coronary artery required treatment, with a diameter of 2.5-3.0 mm and a lesion length of 15-32 mm[23]. At 8 months, the minimal lumen diameter was significantly higher with SES than with control stents (2.22 vs 1.33 mm, $p < 0.0001$). The rate of binary restenosis was significantly reduced with SES compared with control stents (5.9 vs 42.3%, $p = 0.0001$). Significantly fewer patients with SES had major adverse cardiac events at 9 months than did controls (8.0 vs 22.6%, $p = 0.0002$), mainly due to a lower need for target lesion revascularization (4.0 vs 20.9%, $p < 0.0001$)[23]. Therefore, SES are better than BMS for the treatment of single long atherosclerotic lesions in a coronary vessel < 3 mm in diameter[23].

The C-SIRIUS trial assessed the safety and effectiveness of SES in treating single *de novo* long lesions in small native coronary arteries compared to an identical BMS[24]. A total of 100 patients were enrolled at eight Canadian sites. The in-stent minimal lumen diameter at 8 months was 2.46 ± 0.37 mm in the SES group compared with 1.49 ± 0.75 mm in the BMS group (a 65% increase, $p < 0.001$). Angiographic restenosis occurred in 1 of 44 SES patients (2.3%, with no in-stent restenosis) and in 23 of 44 BMS patients (52.3%, $p < 0.001$)[24]. On the basis of these results the investigators could conclude that in patients with long lesions in small vessels, SES reduce the risk of restenosis at 8 months, translating into an excellent clinical outcome at 9 months[24].

The SES-SMART trial was a randomized, multicenter, single-blind, prospective trial performed with 257 patients undergoing percutaneous coronary revascularization for ischemic heart disease, and who had a previ-

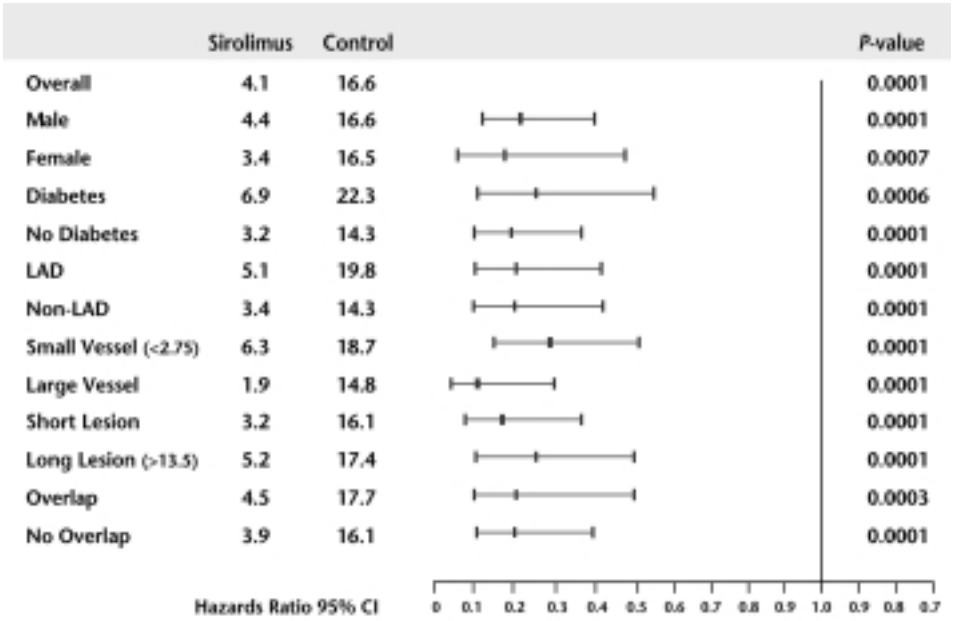

Figure 3. *Odds ratios for target lesion revascularization, by subgroup, in the SIRIUS trial at 9-month follow-up. CI = confidence interval; LAD = left anterior descending coronary artery. Adapted from Perin[27].*

ously untreated atherosclerotic lesion located in a small segment of ≤ 2.75 mm in diameter, in 20 Italian centers between August 2002 and December 2003[25]. Patients were randomly assigned to receive a SES (129 patients) or an uncoated stent having an identical architecture and radiographic appearance (128 patients). The primary endpoint was the 8-month binary in-segment restenosis rate; secondary endpoints included procedural success and the 8-month rate of major adverse cardiac and cerebrovascular events. After 8 months, the binary in-segment restenosis rate was 53.1% (60/113) in the patients receiving an uncoated stent and 9.8% (12/123) in those receiving a SES ($p < 0.001$). Fewer patients randomized to SES experienced major adverse cardiac events (12/129 [9.3%] vs 40/128 [31.3%], $p < 0.001$) mainly because of a reduction in target lesion revascularization (9/129 [7%] vs 27/128 [21.1%], $p = 0.002$) and myocardial infarction (2/129 [1.6%] vs 10/129 [7.8%], $p = 0.04$)[25]. Thus, the use of SES to treat atherosclerotic lesions in small coronary arteries reduces restenosis and may also reduce major adverse cardiac events[25].

Finally, a *post hoc* analysis was performed in 225 patients who received SES in the pooled cohorts of the E-SIRIUS and C-SIRIUS trials[26]. Direct SES deployment performed at the investigator's discretion was as safe and efficacious at mid-term follow-up as stenting preceded by lesion predilation[26].

The paclitaxel-eluting stents. The efficacy of paclitaxel-eluting stents (PES) was compared with that of BMS in a number of randomized, double-blind, multicenter trials in patients with *de novo* coronary artery lesions[27]. The TAXUS I and II trials used the NIR stent, while the pivotal TAXUS IV trial used the Express stent[27]. The primary endpoints of the TAXUS II and IV trials indicated superiority for the PES over the BMS. TAXUS II was a randomized, double-blind trial of 536 patients evaluating slow-release (SR) and moderate-release (MR) formulations of a polymer-based PES (Taxus) for revascularization of single, primary lesions in native coronary arteries[28]. Cohort I compared Taxus-SR with control stents, and cohort II compared Taxus-MR with a second control group. The primary endpoint was 6-month percent in-stent net volume obstruction. Secondary endpoints were 6-month angiographic restenosis and 6- and 12-month incidence of major adverse cardiac events, a composite of cardiac death, myocardial infarction, and repeat revascularization. At 6 months, the percent net volume obstruction within the stent was significantly lower for Taxus stents (7.9% SR and 7.8% MR) than for respective controls (23.2 and 20.5%, $p < 0.0001$ for both)[28]. The incidence of major adverse cardiac events at 12 months was significantly lower ($p = 0.0192$) in the Taxus-SR (10.9%) and Taxus-MR (9.9%) groups than in controls (22.0 and 21.4%, respectively), predominantly because of a significant reduction in repeat target lesion revascularization in Taxus-treated patients[28].

The TAXUS IV study was the largest trial on the paclitaxel-eluting Taxus stent with published and available results at 1 year[29]. In the TAXUS IV trial, 1314 patients with single *de novo* coronary lesions 10 to 28 mm in length, with a reference vessel diameter 2.5 to 3.75 mm, coverable by a single study stent, were prospectively randomized to the SR, polymer-based, paclitaxel-eluting Taxus stent or an identical-appearing BMS. By actuarial analysis, the Taxus stent compared with the BMS reduced the 12-month rates of target lesion revascularization by 73% (4.4 vs 15.1%, $p < 0.0001$), target vessel revascularization by 62% (7.1 vs 17.1%, $p < 0.0001$), target vessel failure by 52% (10.0 vs 19.4%, $p < 0.0001$), and composite major adverse cardiac events by 49% (10.8 vs 20.0%, $p < 0.0001$)[29]. The 1-year rates of cardiac death (1.4 vs 1.3%), myocardial infarction (3.5 vs 4.7%), and subacute thrombosis (0.6 vs 0.8%) were similar between the paclitaxel-eluting and control stents, respectively[29]. Between 9 and 12 months, there were significantly fewer myocardial infarctions (0 vs 1.1%, $p = 0.007$), target vessel revascularizations (2.4 vs 5.8%, $p = 0.002$), and major adverse cardiac events (2.4 vs 6.3%, $p = 0.0009$) in the PES than in the control-stent group, respectively[29] (Fig. 4).

Recently, a small non-randomized trial was conducted to assess the efficacy of PES in chronic total coronary occlusions[30]. In 48 consecutive patients, paclitaxel-eluting Taxus stents were implanted after successful recanalization of a chronic total coronary occlusion (duration > 2 weeks). They were compared with 48 lesion- and risk-matched patients with chronic total coronary occlusions treated with BMS. The 1-year major adverse cardiac event rate was 12.5% in the Taxus group, and 47.9% in the BMS group ($p < 0.001$), which was due to a reduced need for repeat revascularization. The angiographic restenosis rate was 8.3% with Taxus vs 51.1% with BMS ($p < 0.001$)[30]. There was only one late reocclusion with Taxus (2.1%) as compared with 23.4% with BMS ($p < 0.005$). As a result of these findings, the authors concluded that the treatment of chronic total coronary occlusions with a PES drastically reduces major adverse cardiac events and restenosis, and almost eliminates reocclusion, which is typically frequent with BMS in chronic total coronary occlusions. These data would suggest that chronic total coronary occlusions could be a preferred indication for DES[30].

The meta-analysis and meta-regression on drug-eluting stents. The production of an overall estimate of the efficacy of DES is challenging, since the individual studies differ according to several characteristics, including the type, dose and mode of drug delivery, length of follow-up, baseline characteristics of the study population, the outcomes measured, and many other methodological aspects. In order to eliminate such a heterogeneity we performed stratified meta-analyses by each outcome of interest and by type of drug (sirolimus or paclitaxel) used[31].

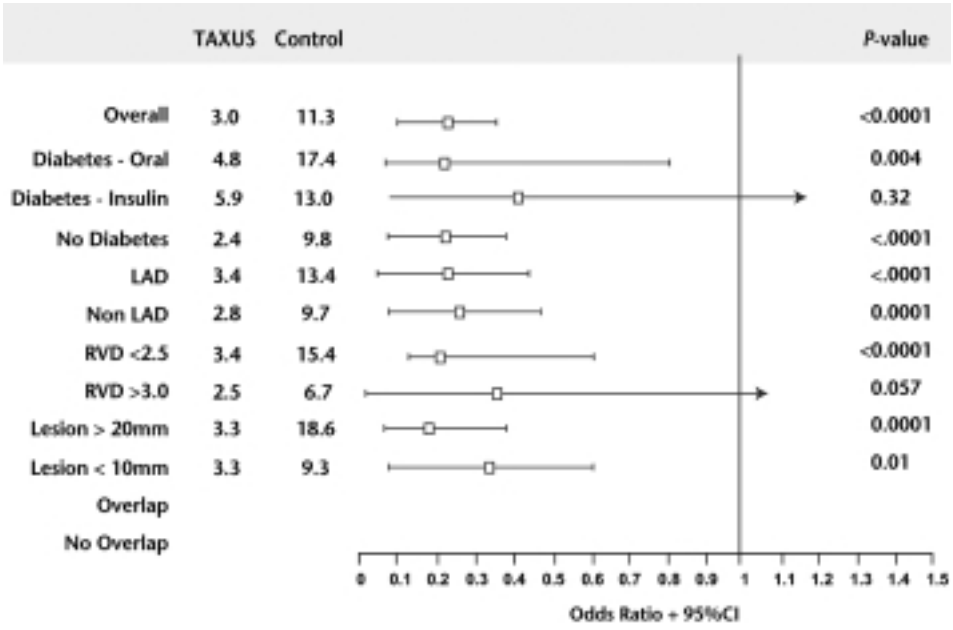

Figure 4. *Odds ratios for target lesion revascularization, by subgroup, in the TAXUS IV trial at 9-month follow-up. CI = confidence interval; LAD = left anterior descending coronary artery; RVD = reference vessel diameter. Adapted from Perin*[27].

Using a meta-analysis approach we demonstrated that DES are better than BMS in reducing the need of additional procedures of revascularization after PCI[31] (Fig. 5). These beneficial effects are not associated with a reduction or an increase in other adverse cardiac events (i.e., death, myocardial infarction, stent thrombosis). Furthermore, these favorable results are sustained during long-term follow-up[1].

Since this meta-analysis demonstrated definitely the superiority of DES vs BMS, it might be speculated that other future studies assessing the efficacy of a new DES should not be anymore tested vs placebo. Perhaps new trials of non-inferiority (or of superiority) vs one of the two available DES should be planned to evaluate new DES.

Overall, it now appears that the use of DES is the increasing preferred method of revascularization for coronary artery disease patients in most circumstances defining the next standard to treat coronary disease. However, it should be pointed out that the superiority of DES vs BMS was demonstrated in the randomized trials in patients with well-defined clinical characteristics. The main common exclusion criteria in these randomized trials were acute myocardial infarction, bifurcational or ostial lesions, unprotected left main, visible thrombus, severe tortuosity and/or calcification, non-native coronary lesions, in-stent restenosis, lesion length > 30 mm. In these conditions, the superiority of DES is still unproven.

In addition, it has been argued that several baseline patients' characteristics, such as presence of diabetes, and severity of disease, measured as length of lesions, degree of stenosis, and type of lesion (C-lesions), could have an impact on the effect of DES. The reported results of the single trials did not allow us to extrapolate data according to subgroups of patients, and in an attempt to verify whether some of these characteristics could have influenced our results, we used meta-regression that, except for the type of drug used, could not identify any variable significantly influencing the results. However, since we believe that the results of a meta-regression should be interpreted cautiously, we strongly recommend that data on subgroups of patients should be made available for analysis.

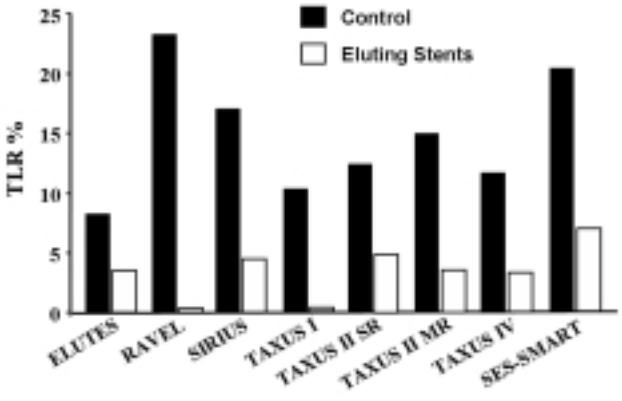

Figure 5. *Target lesion revascularization (TLR) in the randomized trials comparing bare metal stents (Control) and drug-eluting stents (Eluting Stents).*

The problems of drug-eluting stents

The coating polymers. Synthetic polymers, like methacrylate (MA) compounds, have been clinically introduced as inert coatings to locally deliver drugs that in-

hibit restenosis after stent. However, several reports indicated the presence of an exacerbated inflammatory response induced by these polymers compared to BMS[32]. In our laboratory, we carried out a study to evaluate the effects of MA coating alone on VSMC growth *in vitro*. MA-coated stents induced a significant decrease in bromodeoxyuridine incorporation compared with uncoated stents at both the low and high concentrations. In VSMC incubated with MA-coated stents, annexin V/propidium iodide fluorescence detection showed a significant increase in apoptotic cells, which was corroborated by the typical DNA laddering. The MA-coated stent induced VSMC growth arrest by inducing apoptosis in a dose-dependent manner[32]. Thus MA is not an inert platform for eluting drugs because it is biologically active *per se*[32]. This effect should be taken into account when evaluating an association of this coating with antiproliferative agents for in-stent restenosis prevention.

Diabetes. Diabetes is one of the main problems of percutaneous coronary revascularization, representing the actual dark side of in-stent restenosis. We studied the effect of balloon injury on neointimal hyperplasia in streptozotocin-induced diabetic rats with or without adjunct insulin therapy[16]. In addition, to study the effect of balloon injury in non-diabetic rats with hyperinsulinemia, pancreatic islets were transplanted under the kidney capsule in normal rats. First, we demonstrated that glucose did not increase VSMC proliferation and migration *in vitro*. In contrast, insulin induced a significant increase in VSMC proliferation and migration in cell cultures[16]. Furthermore, in VSMC culture, insulin increased MAPK activation. Surprisingly, a reduction in neointimal hyperplasia was documented consistently after vascular injury in hyperglycemic streptozotocin-induced diabetic rats *in vivo*, whereas insulin therapy significantly increased neointimal hyperplasia in these rats[16]. Interestingly, after experimental balloon angioplasty in hyperinsulinemic non-diabetic islet-transplanted rats, a significant increase in neointimal hyperplasia was also observed[16].

Therefore, hyperinsulinemia through activation of the *ras*-MAPK pathway, rather than hyperglycemia *per se*, appears to be crucial in determining the exaggerated neointimal hyperplasia after balloon angioplasty in diabetic animals.

It is worth noting at this point that the results of DES on restenosis prevention are still missing or not conclusive at the most in the setting of diabetic patients, especially when related to insulin-dependent diabetes. In fact, among patients receiving SES, there remains a trend toward a higher frequency of repeat intervention in diabetic patients compared with non-diabetic patients, particularly in insulin-requiring patients[33].

Re-endothelialization and late thrombosis. Although the safety profiles of coronary stents eluting sirolimus or paclitaxel do not seem to differ from those of BMS in the short-to-medium term, concern has arisen about the potential for late stent thromboses related to delayed endothelialization of the stent struts. In fact, molecules like sirolimus and paclitaxel employed for in-stent restenosis prevention have the main target of VSMC proliferation inhibition. Unfortunately, they do not have cell-specific effects as they are cell cycle inhibitors of multiple cell type, including endothelial cells. This turns into the predictable and obvious delay of re-endothelialization of the stented segments when compared to the BMS. The consequence of the described phenomenon is the need to prolong antiplatelet therapy to patients receiving DES. Recently, 4 cases of angiographically confirmed stent thrombosis that occurred late after elective implantation of polymer-based PES (343 and 442 days) or SES (335 and 375 days) were reported[34]. Eventually, all the patients suffered myocardial infarction and, interestingly, all cases arose soon after antiplatelet therapy was interrupted[34]. If confirmed in systematic long-term follow-up studies, these findings are meant to have potentially serious clinical implications. Therefore, more clinical investigations are warranted on this highly critical issue.

Patient selection. Patient selection for DES is still an evolving issue. On the basis of the published randomized data, DES should only be utilized in patients with short, *de novo* coronary lesions[20]. Long-term follow-up as well as experience in more complex lesions is now accumulating and the initial reports that we have described above are still on small number of patients, precluding any factual conclusions. Thus, indications for DES might expand considerably in the near future, but large randomized trials are warranted to further broaden clinical indications.

Moreover, the financial burden of DES will have a major impact on patient selection. Substantial costs may limit utilization of DES to patients at high risk for restenosis. Unfortunately, these patients may require multiple stents, which ultimately increase the cost of the procedure[35]. We are still expecting a more comprehensive answer to this decisive issue.

Aging. Aging is a major risk factor for the development of vascular diseases, such as hypertension and atherosclerosis[36]. Because the prevalence of coronary artery disease is higher in older patients, many of these subjects are candidates for PCI. To this regard, following acute myocardial infarction, PCI, compared to thrombolytic therapy, seems to be the revascularization strategy of choice in elderly patients[36]. Earlier clinical studies have reported a low rate of procedural success and high rates of major complications in older patients undergoing balloon angioplasty[36]. Risks to elderly patients undergoing PCI are 2- to 4-fold higher than those to younger patients, and these are strongly influenced by comorbidities[36]. Data on the safety, efficacy and clinical outcomes of coronary stenting in the

elderly are still limited, even if age-related risk for PCI has decreased as a result of the stent era[36]. In our cath lab, we assessed the effects of balloon injury on neointimal hyperplasia (regarded as *in vivo* VSMC proliferation) and vascular remodeling (involving cell apoptosis and endothelial regeneration) in aged compared to adult rats. This study demonstrated a reduced *in vivo* neointima formation after balloon injury in aged compared to adult animals. This reduced neointimal tissue formation was associated with a worsening of negative arterial remodeling and a reduced endothelial regeneration. These effects seem to be mediated by a decreased function of Akt/endothelial nitric oxide synthase-dependent signaling with aging. As mentioned above, the stent era seems to have improved the outcome of PCI in aged patients[36]. This preliminary finding could correlate with a decreased VSMC proliferation after stent deployment in elderly patients. The results of our study are consistent with these findings as we have demonstrated a decreased VSMC with aging. This is of particular interest in the setting of eluting stents. In fact, if our results could be extrapolated to a clinical scenario, they would question the use of eluting stents in the elderly because of the reduced neointimal formation seen with aging. Furthermore, in the present study we demonstrated a strikingly reduced re-endothelialization in aged rats after balloon injury, which in turn could determine an increased risk for thrombotic complications in aged patients after PCI, especially when using DES that also inhibit endothelial growth. Nevertheless, it is really hard and highly speculative to merge experimental findings into a clinical scenario and, therefore, further appropriate clinical studies should provide additional answers to this problem.

The future of drug-eluting stents

The new field of nanotechnology, opened up by rapid advances in science and technology, creates myriad new opportunities for advancing medical science and disease treatment. In the near future, nanotechnology will play an increasingly significant role in the everyday practice of cardiologists, pulmonologists, and hematologists. Both nanotechnology and nanoscience focus on materials at the atomic, molecular, and supramolecular level, aiming to control and manipulate these new materials by precisely configuring atoms and molecules, producing novel molecular assemblies and designing systems of self-assembly to create supramolecular devices on the scale of an individual cell.

The current generation of DES for the treatment of coronary heart disease is largely based on molecules, which have no cell specificity. In fact, their effects are confined not only to the VSMC compartment but also to the endothelial cells. Common deleterious consequences of this effect are the delay in the re-endothelialization of the stented arterial segment that creates the potential of late thrombosis which requires the long-term use of antiplatelet agents. Nanotechnology-based delivery systems could mitigate these problems by combining tissue- or organ-specific targeting with therapeutic action.

Research has already shown that drugs can be encapsulated in nanospheres or erodible self-assembled structures[37] or conjugated to well-defined multivalent macromolecules such as dendrimers (highly branched polymers)[38]. These mechanisms can improve bioavailability and enable continued release, thereby controlling the initial dose, improving effectiveness, and widening the therapeutic window.

Nanoscale synthetic biomaterials show great promise as scaffolding for regeneration of damaged cellular membranes, tissues, and bones. For example, patterned substrates prepared by microcontact printing can direct *in vitro* generation of differentiated micropatterned endothelial cells.

The above methodologies are still in their infancy age. Therefore, the constant progress of basic as well as translational research will provide us in the next future with more and more sophisticated tools for the development of the ideal DES.

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