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Abstract: Drug-eluting stents (DES) are designed to release pharmacological agents into the vessel wall in order to inhibit the response to injury causing restenosis, i.e., vascular smooth muscle cell migration and proliferation. Once deployed, however, these substances exert many biological effects in the coronary circulation; their action is indeed not confined to inhibition of vascular smooth muscle cells, but extends to other cell types such as endothelial cells. Both rapamycin and paclitaxel decrease endothelial cell migration and proliferation; moreover, they induce tissue factor expression through specific interaction with signal transduction mediators. As both effects would lead to an increased thrombogenic potential of DES, they appear particularly important in light of a possibly increased risk for stent thrombosis with DES as compared to bare-metal stents. This aspect is further highlighted by the observation that DES also decrease proliferation, differentiation, and homing of endothelial progenitor cells, which are believed to contribute to reendothelialization after stent implantation. Furthermore, both rapamycin and paclitaxel have been demonstrated to induce endothelial dysfunction in the coronary vasculature distal to the stent. Finally, the polymer used for DES may be associated with hypersensitivity reactions, which may, at least in some cases, favor stent thrombosis. This review will discuss the biological effects of DES in the coronary vasculature

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Biological Effects of Drug-Eluting Stents in the Coronary Circulation

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Abstract
Drug-eluting stents (DES) are designed to release pharmacological agents into the vessel wall in order to inhibit the response to injury causing restenosis, i.e., vascular smooth muscle cell migration and proliferation. Once deployed, however, these substances exert many biological effects in the coronary circulation; their action is indeed not confined to inhibition of vascular smooth muscle cells, but extends to other cell types such as endothelial cells. Both rapamycin and paclitaxel decrease endothelial cell migration and proliferation; moreover, they induce tissue factor expression through specific interaction with signal transduction mediators. As both effects would lead to an increased thrombogenic potential of DES, they appear particularly important in light of a possibly increased risk for stent thrombosis with DES as compared to bare-metal stents. This aspect is further highlighted by the observation that DES also decrease proliferation, differentiation, and homing of endothelial progenitor cells, which are believed to contribute to reendothelialization after stent implantation. Furthermore, both rapamycin and paclitaxel have been demonstrated to induce endothelial dysfunction in the coronary vasculature distal to the stent. Finally, the polymer used for DES may be associated with hypersensitivity reactions, which may, at least in some cases, favor stent thrombosis. This review will discuss the biological effects of DES in the coronary vasculature.

Biologische Effekte von Drug-eluting-Stents im koronaren Gefäßbett

Schlüsselwörter:
Drug-eluting-Stents · Restenose · Thrombose · Tissue-Faktor · Endotheliale Regeneration

Zusammenfassung
Drug-eluting-Stents (DES) setzen nach ihrer Implantation pharmakologische Substanzen in die Gefäßwand frei, welche die zur Restenose führenden Prozesse – Migration und Proliferation glatter Gefäßmuskelzellen – inhibieren. Die Wirkung dieser Pharmaka ist jedoch vielfältig und zudem nicht auf glatte Gefäßmuskelzellen beschränkt; so inhibieren sowohl Rapamycin als auch Paclitaxel die Migration und Proliferation von Endothelzellen und induzieren außerdem die Expression von Tissue-Faktor durch spezifische Interaktion mit Mediatoren der Signaltransduktion. Da diese beiden Effekte zu einer erhöhten Stentthrombogenität führen können, erscheinen sie von großer Wichtigkeit, insbesondere im Rahmen der aktuellen Diskussion um eine möglicherweise erhöhte Rate von Stentthrombosen bei DES im Vergleich zu unbeschichteten Bare-Metal-Stents. Hinzu kommt, dass sowohl Rapamycin als auch Paclitaxel die Proliferation, Migration und Differenzierung von endothelialen Progenitorzellen, welche ebenfalls zur Reendothelialisierung nach Stentimplantation beizutragen scheinen, hemmen. Darüber hinaus konnte gezeigt werden, dass beide Substanzen in Gefäßsegmenten distal der gestenteten Läsion eine endotheliale Dysfunktion induzieren. Schließlich kann das Polymer, welches zur Beschichtung verwendet wird, zu Hypersensitivitätsreaktionen in der Gefäßwand führen, was zumindest in manchen Fällen die Entstehung einer Stentthrombose zu begünstigen scheint. Dieser Übersichtsartikel beleuchtet die biologischen Effekte von DES im koronaren Gefäßbett.

Introduction

With the introduction of balloon-expandable bare-metal stents (BMS), coronary remodeling and in turn restenosis rates were reduced as compared to angioplasty alone [16, 44]. As the risk of restenosis remained > 10%, however, drug-eluting stents (DES) were designed which release pharmacological agents after deployment. On first-generation DES, rapamycin (sirolimus, used on Cypher® stents) or paclitaxel (used on Taxus® stents) were applied in order to reduce restenosis through inhibition of vascular smooth muscle cell (VSMC) migration and proliferation. As a result, the rates of restenosis and target vessel revascularization could be reduced to < 10% after DES implantation [37, 38].

Already in the first series of patients receiving BMS, however, stent thrombosis was recognized as a severe complication owing to its high mortality. Indeed, despite reduced restenosis rates, the incidence of in-stent thrombosis has not decreased with DES as

compared to BMS [3, 4, 8, 35, 36]. Several hundred cases of stent thrombosis have been reported for rapamycin-coated stents [32], and a number of reports imply that thrombosis rates of DES may even be higher in "real-world" patients than in clinical trials [27, 40].

This review article focuses on the biological effects of DES in the coronary circulation.

Biological Effects of Drug-Eluting Stents in the Coronary Circulation

DES Impair VSMC Migration and Proliferation

Agents released from DES inhibit migration and proliferation of VSMCs, which are the crucial events in neointima formation and the development of restenosis (Figure 1) [33, 42]. In vitro, VSMC proliferation is blocked by rapamycin via interference with several cell-cycle regulators involved in G_1–S phase transition including $p27^{Kip1}$ [31], $p21^{cip1}$ [30], cyclin D isoforms, and, finally, the retinoblastoma protein (pRb) [33]. Although not fully resolved in detail, the antimigratory effect of rapamycin appears to occur via interaction with $p27^{Kip1}$ as well [50]. In vivo, rapamycin inhibits intimal thickening after balloon angioplasty in porcine and rodent animal models through interaction with cell-cycle regulators [17, 43]. The role of $p27^{Kip1}$ in this process, however, remains unclear, as some studies imply an effect of rapamycin on this protein [17], while others do not [6, 43].

Everolimus, an orally active compound of the sirolimus family, is used on second-generation DES (Xience®); when applied orally in a rabbit iliac artery model, it significantly reduces neointimal growth [14]. Similarly, zotarolimus, a synthetic rapamycin analog developed specifically for stent application (Endeavor®), inhibits VSMC migration and proliferation and reduces neointima formation in a porcine coronary artery model [18].

Paclitaxel, a lipophilic diterpenoid that binds to the β-subunit of the tubulin heterodimer, stabilizes microtubules, induces cell-cycle arrest in G_2–M phase, and, eventually, inhibits VSMC migration and proliferation [2, 11, 45]. Consistently, an inhibition of neointima formation is observed in animal models after local delivery of paclitaxel [25].

DES Inhibit Endothelial Cell Migration and Proliferation

Coronary angioplasty leads to disruption of the endothelial layer leaving a highly prothrombotic surface exposed to the blood stream. Restoration of an intact endothelium therefore represents a crucial process in reestablishing an antithrombotic vessel surface. Traditionally, it is believed that reendothelialization occurs after vascular injury and stent deployment because endothelial cells proliferate and migrate from intact neighboring coronary segments, eventually leading to endothelial healing of the injured segment.

Given the ubiquitous expression of cell-cycle regulatory proteins, it is conceivable that agents released from DES do not only affect proliferation and migration of VSMCs, but also of endothelial cells (Figure 1). Indeed, rapamycin potently inhibits endothelial cell proliferation [48] and migration [34] in vitro. Similarly, paclitaxel reduces endothelial cell proliferation and migration through interaction with cell-cycle regulators and, at least in part, through induction of apoptosis [41].

In animal models, the time course of reendothelialization after stent implantation varies in different species. In pigs, the extent of reendothelializa-

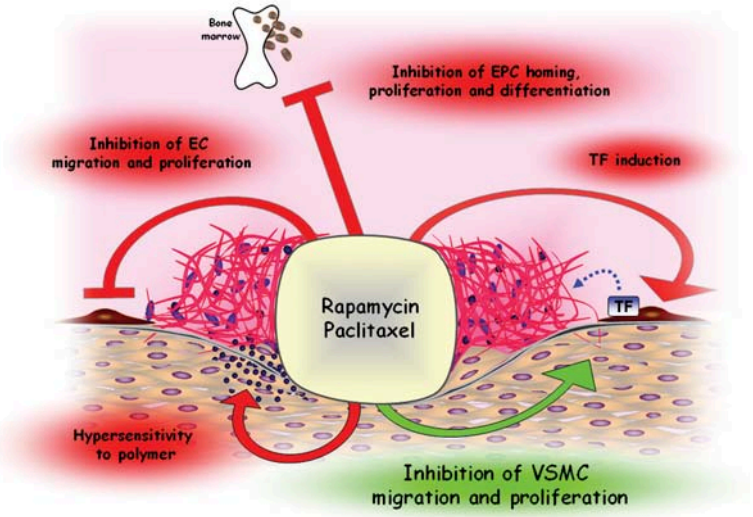
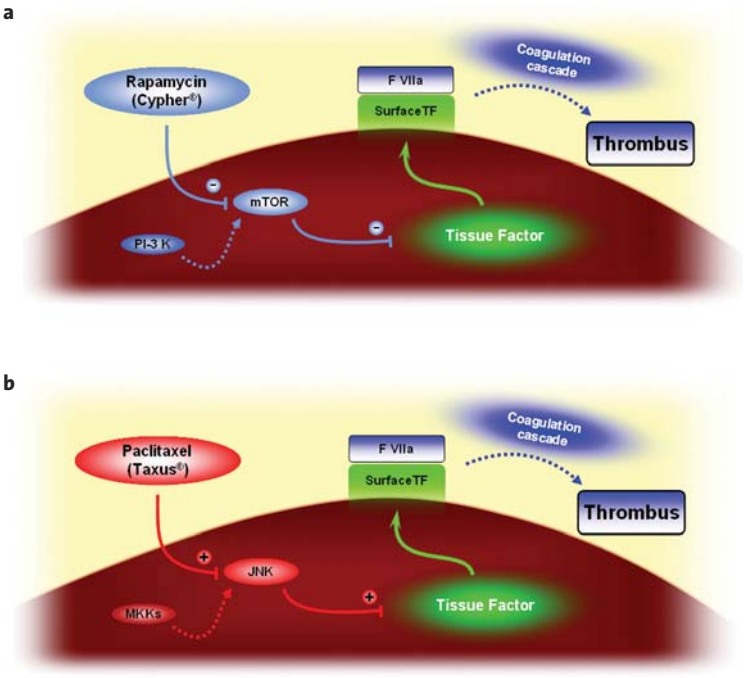


Figure 1. Biological effects of drug-eluting stents (DES) in the coronary circulation.
Simplified overview of the effect of a DES strut on the vessel wall after deployment. Sirolimus/paclitaxel inhibit vascular smooth muscle cell (VSMC) migration and proliferation, thereby reducing restenosis. On the other hand, these drugs inhibit migration and proliferation of neighboring endothelial cells (EC), induce tissue factor (TF) expression, and inhibit homing, proliferation, and differentiation of endothelial progenitor cells (EPC), potentially leading to an increase in stent thrombogenicity. Moreover, the polymer or the stent strut itself may elicit a hypersensitivity reaction in the vessel wall.

Abbildung 1. Biologische Effekte von Drug-eluting-Stents (DES) in der Koronarzirkulation.
Vereinfachter Überblick über den Effekt von DES auf die Gefäßwand nach Implantation. Sirolimus/Paclitaxel inhibieren die Migration und Proliferation glatter Gefäßmuskelzellen (VSMC) und reduzieren somit die Restenose. Auf der anderen Seite inhibieren diese Medikamente ebenfalls die Migration und Proliferation von angrenzenden Endothelzellen (EC); des Weiteren induzieren sie die Expression von Tissue-Faktor (TF) und inhibieren das „homing", die Proliferation sowie die Differenzierung endothelialer Vorläuferzellen (EPC), was potentiell die Stentthrombogenität erhöht. Darüber hinaus kann das Polymer oder das Stentgerüst selbst eine Hypersensitivitätsreaktion in der Gefäßwand auslösen.



Figures 2a and 2b. Rapamycin and paclitaxel increase tissue factor (TF) expression.
a) Rapamycin binds to the mammalial target of rapamycin (mTOR); mTOR is a downstream mediator of the phosphatidylinositol-3 kinase (PI-3K) pathway, which plays an inhibitory role in TF expression. Hence, by inhibiting mTOR, rapamycin leads to an increase in TF protein expression and surface activity. b) Paclitaxel increases c-jun NH2-terminal kinase (JNK) activation leading to an enhanced TF protein expression as well as surface activity. Thus, paclitaxel directly activates a positive regulatory pathway of TF expression, while rapamycin upregulates TF by disinhibiting an inhibitory pathway. MKKs: MAP kinase kinases.

Abbildungen 2a und 2b. Rapamycin und Paclitaxel erhöhen die Expression von Tissue-Faktor (TF).
a) Rapamycin bindet an mTOR („mammalial target of rapamycin"); mTOR ist ein Downstream-Mediator des Phosphatidylinositol-3-Kinase-(PI-3K-)Signaltransduktionswegs, welcher eine inhibitorische Rolle in der TF-Induktion spielt. Über die Hemmung von mTOR führt Rapamycin somit zu einer Zunahme der TF-Proteinexpression und Oberflächenaktivität. b) Paclitaxel verstärkt die Aktivierung von JNK („C-jun NH2-terminal kinase"), welche zu einer Erhöhung der Proteinexpression und Oberflächenaktivität von TF führt. Paclitaxel aktiviert somit direkt einen positiven Signaltransduktionsweg, während Rapamycin TF durch die Disinhibition eines hemmenden Signaltransduktionswegs induziert. MKKs: „MAP kinase kinases".

tion is similar between BMS and DES after 28 days [51]; however, delayed endothelial healing occurs in a rabbit iliac overlapping stent model after implantation of sirolimus- or paclitaxel-eluting stents as compared to BMS [15]. In humans, near complete reendothelialization seems to occur by 3–4 months after BMS implantation [13]. In an autopsy study comparing coronary artery segments after implantation of DES with BMS, delayed arterial healing and poorer reendothelialization are documented after DES implantation [29]. Hence, there is evidence that the currently used DES may significantly impair reendothelialization after coronary artery stent deployment.

DES Inhibit Homing, Proliferation, and Differentiation of Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) seem to be involved in reendothelialization after angioplasty [19, 53]; hence, it is important to understand the effect of DES on EPC biology. Rapamycin indeed inhibits proliferation, migration, and differentiation of human EPCs in vitro [7, 10]; these effects occur via interaction with the mammalian target of rapamycin (mTOR) and, at least in part, by induction of apoptosis. It therefore comes as no surprise that, in contrast to BMS, implantation of a sirolimus-eluting stent leads to a decrease in circulating CD34-positive cells [28]. Hence, drugs loaded on DES may affect homing, proliferation, and differentiation of EPCs thereby delaying proper endothelial regeneration (Figure 1).

DES Induce Endothelial Tissue Factor Expression

On a molecular level, rapamycin binds to the FK-binding protein 12 (FKBP-12) and thereby inhibits phosphorylation of mTOR. mTOR is a downstream target of the phosphatidylinositol-3 kinase (PI-3K) pathway, which in turn regulates translation by phosphorylation of p70S6 ribosomal protein kinase (p70S6K) [24]. The inhibitory role of the PI-3K pathway on expression of endothelial tissue factor (TF) is well established, as its inhibition enhances TF expression in response to thrombin or tumor necrosis factor-(TNF-)α [9, 12]. Consistently, inhibition of mTOR by rapamycin increases thrombin- as well as TNF-α-induced endothelial TF expression and activity (Figure 2a) [48]. Importantly, this effect occurs at rapamycin concentrations observed in vivo after stent implantation [48, 54]. The effect of rapamycin on TF expression in VSMCs is controversial: while we did not observe an effect after 5 h of incubation [48], prolonged exposition (e.g., 24 h) of rapamycin has been demonstrated to slightly elevate VSMC TF expression [57].

Paclitaxel does not only stabilize microtubules, but also activates signal transduction molecules such as JNK [46, 55], an important mediator of endothelial TF induction [20, 47, 49]. Consequently, paclitaxel increases endothelial TF expression and activity (Figure 2b) [46]; again, the concentrations exerting this effect are comparable with local tissue concentrations encountered after stent deployment [15].

Sirolimus-eluting stents are designed in a way that about 80% of the loaded rapamycin has eluted by 30 days [37, 38]. By contrast, paclitaxel-eluting

stents have a biphasic drug release profile in vitro with an initial burst during the first 48 h after implantation followed by a sustained low-level release for at least 2 weeks [23]. Owing to their lipophilic properties, both rapamycin and paclitaxel easily penetrate cell walls leading to retention of the drugs in the arterial tissue [15, 21, 51]. Thus, TF induction in response to both rapamycin and paclitaxel may promote a prothrombotic environment after deployment of DES (Figure 1). In how far these findings truly translate into clinical practice, however, requires further studies, in particular with respect to the extent and the spatiotemporal pattern of TF induction in the arterial wall after DES deployment.

DES Induce Endothelial Dysfunction in the Distal Coronary Circulation

There is evidence that both rapamycin and paclitaxel may not only affect the vasculature at the site of stent implantation, but also in coronary artery segments distal to the stent. Indeed, long-term endothelial dysfunction of coronary artery segments distal to sirolimus-eluting stent, but not BMS, occurs as indicated by significant vasoconstriction in response to acetylcholine infusion [26]. Similarly, implantation of paclitaxel-eluting stents is associated with exercise-induced vasoconstriction in the segments proximal and distal to the stent, while exercise-induced vasodilation is observed after BMS placement [52]. Taken together, these findings indicate that drugs released from DES may affect the distal coronary vasculature facilitating coronary vasoconstriction and potentially contributing to no-reflow phenomena after stent deployment.

Effect of the Stent Material in the Coronary Circulation

Certain stent materials appear to promote the development of stent thrombosis. For example, implantation of an open-cell versus a closed-cell stent resulted in greater platelet activation 30 days following implantation [22]. The stent strut thickness as well as the polymer type also appear to play an important role. Chronic eosinophilic infiltration of the arterial wall suggestive of a hypersensitivity reaction has also been observed, probably in response to the nonerodable polymers of DES [29, 39]; mostly, these reactions occur > 4 months after DES implantation (Figure 1) [29, 32]. While the polymer may have an effect on the coronary vasculature after DES placement, the causal relationship between polymer-induced inflammation and the incidence of late stent thrombosis has only been suggested in a minority of patients.

Design of Future Drug-Eluting Stents

The ideal DES inhibits restenosis without interfering with (or even expediting) reendothelialization, all on the basis of a biologically inert polymer. While several novel approaches aim at improving currently available DES, no ideal combination of materials and drugs has yet been developed. One potential approach uses a simple chemical coating, such as titanium-nitride oxide, which diminishes platelet adhesion and fibrinogen binding as compared to BMS; whether this strategy will be effective against restenosis and stent thrombosis, however, remains to be studied in large clinical trials [56]. Coating of stents with agents facilitating reendothelialization after stent implantation may represent another feasible approach to reduce the thrombogenity of DES. In a porcine coronary artery stenting model, stents loaded with an integrin-binding cyclic Arg-Gly-Asp peptide indeed accelerate endothelialization by attracting EPCs [5]. In humans, endothelial coverage is accelerated after implantation of stents coated with antibodies against CD34 designed to capture EPCs [1]. However, further studies are needed to assess the long-term efficacy and safety of these biologically active stents. A combination of substances accelerating endothelial healing (such as vascular endothelial growth factor and others) with established agents reducing restenosis represents another promising approach.

Dimethyl sulfoxide (DMSO) may also represent an interesting therapeutic principle for DES coating. We recently demonstrated that DMSO prevents VSMC proliferation and migration; at the same time, DMSO reduces upregulation of TF in endothelial cells, vascular smooth muscle, and monocytes, and prevents thrombotic occlusion in vivo in a mouse carotid artery injury model [9]. Thus, DMSO may have the potential to inhibit both neointima formation and stent thrombosis, if loaded on a DES. Whether these promising first results hold true in a larger animal model and in humans, however, remains to be determined.

Summary and Conclusion

Once deployed, DES exert different biological effects in the coronary circulation. Importantly, their action is not confined to inhibition of migration and proliferation of VSMCs, i.e., important factors in neointima formation and the development of restenosis, but extends to other cell types such as endothelial cells. Indeed, both rapamycin and paclitaxel decrease endothelial cell migration and proliferation; in addition, the drugs induce endothelial TF expression through specific interaction with signal transduction mediators. Furthermore, DES decrease homing, proliferation, and differentiation of EPCs, equally believed to be involved in endothelial regeneration af-

ter stent implantation. Both rapamycin and paclitaxel have also been demonstrated to induce endothelial dysfunction in the distal coronary vasculature. Finally, the polymer used for DES may be associated with hypersensitivity reactions which may, at least in some cases, promote stent thrombosis.

These undesired local side effects of DES in the vessel wall clearly demonstrate the need for improvement of currently applied first-generation DES; indeed, these side effects appear particularly important in the light of the ongoing debate of a possibly increased risk of stent thrombosis with DES as compared to BMS. Hence, improvements of currently available systems as well as the introduction of novel treatment strategies are needed in order to advance the development toward the goal of preventing restenosis without the cost of delayed endothelial healing and increased stent thrombogenity.

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