Drug-eluting stents show delayed healing: paclitaxel more pronounced than sirolimus

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Aims To understand wound healing after drug-eluting stents (DES) placement in humans, we studied the histology of in-stent restenosis (ISR) tissue obtained by atherectomy from bare metal stents (BMS) and DES in comparison with de novo atherosclerosis.

Methods and results The tissue was retrieved from ISR in ten sirolimus-eluting stents (SES) and nine paclitaxel-eluting stents (PES), six BMS, and nine stenotic de novo atherosclerotic lesions and processed for histology and immunocytochemistry. Patients with ISR in PES showed a significantly higher incidence of unstable angina upon presentation for re-intervention ($P = 0.046$). De novo tissue tended to be more collagen rich, whereas ISR tissue tended to be more proteoglycan rich. In all groups, cell content consisted almost exclusively of smooth muscle cells. Histology showed that fibrinoid in ISR tissue was present only in DES ($P = 0.004$), as late as 2 years following DES placement, indicating a persistent incomplete healing response. The amount of fibrinoid, given as a percentage of total tissue in each atherectomy specimen, was greater in PES than in SES ($17\%$ vs. $5\%$, $P = 0.026$).

Conclusion ISR in DES shows incomplete neointimal healing as late as 2 years after implantation. Patients with ISR in PES presented with more unstable angina and showed more pronounced signs of delayed healing than SES.

Introduction

Drug-eluting stents (DES) loaded with sirolimus and paclitaxel have shown superior angiographic and clinical results compared with bare metal stents (BMS) in patients with coronary artery disease undergoing PCI. Sirolimus, developed as an antibiotic with potent immunosuppressive properties, blocks cell cycle progression from G1- to S-phase as well as cell migration. This is effectuated through its binding to FKBP12, which inhibits mTOR, up regulates p27kip-1, and as a result, halts cell cycle progression. Paclitaxel, developed as an anti-neoplastic drug, inhibits the disassembly of microtubules which blocks mitosis progression and thus G2/M-phase arrest. Halting cell cycle progression is thought to be the main mode of action in reducing neointimal thickening following stenting. Indeed, DES decrease late loss and restenosis, as reported in randomized trials and routine clinical practice. However, in-stent restenosis (ISR) in DES still occurs, although to a limited extent. The purpose of the present study was to compare the lesion characteristics (histology, angiography) of ISR tissue obtained by means of atherectomy from BMS, sirolimus-eluting stents (SES) and paclitaxel-eluting stents (PES).

Methods

Population

The tissue was retrieved from ten SES, nine PES, six BMS and nine de novo lesions during the course of the RESEARCH and T-SEARCH registries. There was no preset selection of patients, and all patients with restenosis and eligible for atherectomy (coronary anatomy: size, tortuosity) were included. Given the 4% revascularization rate in the patient group, the total number of DES atherectomies amounted to ~50% of all restenosis lesions enrolled during the sampling period. Only patients presenting with late stent thrombosis were not included in the analysis. Patient and lesion demographics are listed in Tables 1 and 2.

Angiographic analysis

Quantitative angiograms taken prior to the atherectomy were analysed offline using QCA (CAAS II, PIE Medical) to determine the angiographic pattern of restenosis according to Mehran et al. Lesion length was assessed by automatic contour detection, as well as by manually corrected length, using a cut-off point of 50%
Tissue retrieval and preparation

Tissues were retrieved by means of standard directional atherectomy (Flexi-cut™, Guidant Europe SA, Diegem, Belgium), fixed in 4% buffered formaldehyde, and embedded in paraffin for histology (Figure 1). Sections were stained with haematoxylin eosin (HE) as a routine stain, picrosirius red (PSR) for collagen, Alcian Blue (AB) for proteoglycans, and with antibodies against smooth muscle cell-specific α-actin (Biogenex, MU 128-UC, clone 1A4, 1:50), p27 (Novo-castra, NCL-p27, clone 1B4, 1:30), common leucocyte antigen (CLA) (Dako, CD45, M0701, clone 2B11; PD7/26, 1:100), proliferating cells (Dako, KI67, M7240, Clone Mib-1, 1:100), and erythrocyte remnants (glycophorin C, Dakocytomation, MO820, 1:200), using horse radish peroxidase and diaminobenzidine as detecting reagents. Tissue fragments containing internal or external elastic laminae (i.e. media or adventitia) were not analysed.

Histological analysis

Tissues were scored as collagen or proteoglycan rich on the basis of PSR, AB, and HE. Inflammation was assessed by HE and CLA. Special attention was paid to the presence of eosinophils. Proliferation was assessed with Mib-1. Tissue components such as atheroma, thrombus, and fibrinoid were scored on morphological grounds. Atheroma was classified as tissue-containing macrophage foam cells, the imprint of cholesterol-crystals or necrotic core. Thrombus was classified as proteinaceous mass containing fibrin, platelets, erythrocytes, or glycophorin C. Old thrombus was classified as infiltrated by smooth muscle cells. Fibrinoid was classified as amorphous and dense proteoglycan mass negative for glycophorin C.

Morphometric analysis

Cell density was assessed in collagen-rich and proteoglycan-rich tissue by counting well-defined nuclei in at least 20 fields at 40× magnification or else in all available tissue. Inflammatory areas were not counted for cell density measurements. Areas were measured using a microscopy image analysis system (Clemex technologies, Inc, Quebec, Canada).

Statistical analysis

Differences between the groups were assessed with SPSS (version 11.0), using the Kruskal–Wallis test (non-parametric test) as an overall test. Only in case of statistical significance was this followed by a Mann–Whitney U test (two-tailed significance). Comparisons between stent groups were of interest. A P-value < 0.05 was considered statistically significant. Linear regression was used to assess the predictive value of patient characteristics in the occurrence of fibrinoid and thrombus.

Results

Patient demographics and angiographic characteristics

Data summarized in Tables 1 and 2 show a similar distribution pattern of patient age, angiographic lesion characteristics, and patient characteristics, except for clinical presentation (SAP, UAP, SI) at the time of atherectomy. Here, PES showed a significantly higher incidence of unstable angina upon presentation for re-intervention (P = 0.046).

Lesion type and length did not differ between the groups, irrespective of analysis technique (automatic vs. manual). Automated contour detection determined the majority of lesions as diffuse, whereas the 50% cut-off point determined the majority as focal.
Histological analysis

Qualitative assessment showed that the atherectomy samples consisted almost exclusively of smooth muscle cells (Figures 2 and 3). Materials from all stent types were highly proteoglycan rich (>60%) as opposed to de novo tissue which was highly fibrotic (53%, Table 3). Smooth muscle cells in all groups showed a low number of proliferating cells, as assessed by Mib-1 (<1%). Expression of p27 in SES was low in neointimal tissue, except for areas with neovascularization: here, the neovascular bed with surrounding inflammatory cells consistently stained positive for p27.

Markers of impaired healing (Table 4), i.e. organized thrombus and fibrinoid, were often found in SES and PES. Fibrinoid could be found as late as 2 years following stenting. Both the incidence (Table 4) and the percentage fibrinoid (the amount of fibrinoid in each sample expressed as a percentage of total tissue in that sample) were significantly different between the groups (respectively, \( P = 0.004 \) and 0.004, Kruskal–Wallis). Pairwise analysis (Mann–Whitney \( U \) test) showed that this percentage was higher in PES than in SES (17 vs. 5%, \( P = 0.026 \)). We investigated the independent predictive value of DES for markers of delayed healing. We used linear regression adjusting for all baseline characteristics, namely, patient age, lesion age, clinical presentation, gender, and diabetes. The effect of DES was independent from these factors (adjusted \( P \)-value = 0.034). No other baseline characteristic predicted delayed healing. Inflammatory areas were scarce in all groups, but these areas showed the highest level of proliferation (Mib-1).

Morphometric analysis

Quantitative data are summarized in Table 3. Although cell density of the fibrotic component was similar in all four groups (448–540/mm²), the proteoglycan-rich component showed large differences in cell density, with the highest density in the de novo group (922 cells/mm²) and the lowest in the SES group (580 cells/mm²). This difference was however not statistically significant (\( P = 0.2 \)).

Discussion

The current study set out to assess the differences between restenosis in BMS and in DES in tissue samples obtained by atherectomy. The main result from the study shows that restenosis in stents differs from de novo lesions in the quantity of proteoglycan-rich tissue. Restenosis in DES differs qualitatively from BMS in the presence of fibrinoid, which is associated with incomplete healing in several tissues. PES showed more fibrinoid (3×) than SES.

Delayed wound healing in drug-eluting stents

Restenosis tissue obtained by atherectomy, after both balloon angioplasty and BMS, shows abundant...
Our data also show similarity between restenosis in BMS and in DES. Predominance of proteoglycans is related to general vascular wound-healing responses. Fibrinoid is a non-collagenous, non-proteoglycan-containing tissue, characterized by a dense and amorphous appearance (fibrin-like) only sparingly infiltrated by cells. It is not the same as old thrombus, as indicated by a lack of staining for erythrocyte remnants (Glyco C). Both fibrinoid and old thrombus have been associated with delayed healing. Systemic sirolimus was associated with fibrinoid vascular necrosis before. Systemic taxol only in conjunction with radiotherapy, although the amorphous acellular material in PES described by Heldman could well be the same. A recent study showed that at sites of overlapping stents in peripheral arteries, delayed healing was also observed and was more intense in paclitaxel than in sirolimus.

Although it could be argued that patient age or lesion age might influence markers of delayed healing, the effect of DES was independent from these factors (adjusted P-value = 0.034).

**Mechanism of restenosis in drug-eluting stents**

Whether delayed healing is linked to restenosis is unknown. Animal experiments indicate that fibrinoid always occurs and is still present at 3 months.

Clinical data suggest that low local drug concentrations, based on local stent geometry, might well be a cause for restenosis. That a declining local drug concentration was also responsible for catch-up/restenosis in our patients seems likely. Another possibility is late polymer toxicity or hypersensitivity, but the fact that restenosis in DES is focal does not make sense in that respect. A diffuse reaction would then be more likely. Moreover, we did not find any incidence of eosinophilic infiltrates in the tissue. We cannot rule out that late stent restenosis is related to a low-grade but chronic irritation of the vessel wall by the polymer coating or its products.

**Hypersensitivity**

A recent paper, based on data from FDA reports and autopsy cases, suggested hypersensitivity in polymer-based DES may result in late thrombosis and death. We lack the numbers to either deny or corroborate this data. In our study, we did not find any incidence of eosinophilic infiltrates in stent tissue, and delayed healing was found even in the absence of eosinophils.

**Focal vs. diffuse in-stent restenosis in drug-eluting stents compared with bare metal stents**

It has been suggested that angiographic ISR is usually focal in nature. The method of lesion length measurement is, however, usually not described. We therefore compared automated and manual lesion length measurements. Manual length, based upon a segment with ≥50% diameter stenosis, varied significantly from automated lesion length, with automatic length predominantly diffuse and manual length predominantly focal (P = 0.0001). There were no differences between the stent groups.

**Late stent malapposition**

It is tempting to associate late acquired stent malapposition (LMA) with fibrinoid, but a clear correlation between IVUS, LMA, and histology has not been described. LMA has been described by some as a pure IVUS finding without clinical repercussions and clinically found to occur in both DES and BMS. The incidence of LMA clearly differs between the stent groups and ranges from 5% in BMS to 14% in DES. Although LMA has been observed histologically, it seems to be restricted to DES use only. This may, however, be the result of the experimental models used to test stents. We need to clearly separate malapposition observed by IVUS from malapposition observed microscopically.

Autopsy studies suggest that LMA is associated with increased risk of late stent thrombosis. The fact that malapposition may be of importance is also illustrated by our own observations (unpublished results) that even in BMS, stent struts not apposed to the wall, e.g. over side branches, are not healed at 28 days. The study of atherectomy specimen cannot however shed light on the association between stent thrombosis and fibrinoid, as the presence of a fibrous cap, impossible to be studied in atherectomy specimen, is of importance, preventing contact between fibrinoid and flowing blood.
Limitations

Although the atherectomy by itself acts as a selection procedure since tortuous and small vessels are unsuitable, it is unclear whether biopsies from smaller and tortuous vessels would affect the outcome. The current study is based on a limited number of observations. The number of bare stent samples is especially low because implantation has dropped dramatically since the introduction of DES. The skewed sex ratio between PES, SES, and BMS may be the result of small numbers. However, in both PES and SES, female gender was shown not to be an independent predictor of MACE and TVR, nor was it an independent predictor of delayed healing in our study.

Conclusion

Restenotic tissue from DES shows persistent signs of delayed or incomplete wound healing compared with BMS, and these are more intense in PES than in SES.

Implications

Our findings may warrant long follow-up periods in clinical studies in order not to miss late catch-up. It also underscores the importance of the current clinical practice of prescribing antplatelet therapy with aspirin and clopidogrel for an extended period of time. Finally, our findings call for the development of stents coated with alternative drugs/therapies that reduce restenosis to a similar extent but without delaying the healing response for such a long time.

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References


