Neointimal Tissue Response at Sites of Coronary Stenting in Humans
Macroscopic, Histological, and Immunohistochemical Analyses

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Background—Experimental animal studies have shown that coronary stenting induces neointimal proliferation. However, the histopathological events after coronary stenting in humans have not been studied systematically.

Methods and Results—We investigated 11 stented coronary arteries (9 Palmaz-Schatz stents, 1 Wiktor stent, and 1 ACS Multi-Link stent) obtained from 11 patients who had died 2 days to 21 months after stenting. We focused on gross, histological, and immunohistochemical aspects of the repair processes. Two patients developed symptoms of restenosis. Serial sections were stained with antibodies against smooth muscle cells (SMCs), macrophages, and endothelial cells. At 9 and 12 days after stenting, the stent sites showed thrombus formation with early formation of neointima composed of abundant macrophages and α-actin-negative spindle cells. From 64 days on, all sites with stenting showed a distinct layer of neointima, albeit to varying degrees. In nonrestenotic lesions, neointimal thickening was markedly less than in restenotic lesions but without qualitative differences; the neointima contained macrophages but was composed predominantly of α-actin-positive SMCs.

Conclusions—These observations strongly support the concept that neointimal proliferation in humans is a process of staged redifferentiation of SMCs, which may cause in-stent stenosis. Moreover, the exuberant neointimal proliferation with accumulation of macrophages and extensive neovascularization at sites of stent restenosis suggests a role for organization of mural thrombus.

Key Words: stents • restenosis • coronary disease • immunohistochemistry

Coronary stenting is the only procedure that has been proven to reduce the incidence of late restenosis after PTCA. However, thus far only sparse data are available in humans regarding the healing processes involved after stent implantation. Indeed, an enormous gap exists between animal experiments on one hand and insights obtained from observations in humans on the other. In fact, to the best of our knowledge, only 4 autopsy case reports exist of patients who had died 12 hours, 15 days, 3 weeks, and 8 weeks, respectively, after implantation of a coronary stent, and in 2 instances, atherectomy specimens, retrieved from restenotic lesions after coronary stenting with a Palmaz-Schatz device, have been reported. Obviously, there is a need for more detailed information in humans.

We are not aware of any systematic studies in humans focusing on gross, histological, and immunohistochemical aspects of the repair processes involved. The present study addresses this need.

Methods

Patients
This study is based on 11 coronary arteries obtained at autopsy from 11 patients who had undergone elective or emergency coronary stenting. The relevant clinical data are summarized in the Table. The mean age of the patients (9 men and 2 women) was 76 ± 6.4 years (range, 66 to 83 years). In 3 patients with acute myocardial infarction (patients 2, 3, and 4), stent implantation was performed to treat coronary dissection after emergency PTCA. One patient (patient 1) with stable angina underwent stenting to treat coronary dissection after PTCA. All 4 patients (patients 1, 2, 3, and 4) showed immediate post-PTCA angiographic evidence of dissection before stent implantation. Two patients (patients 7 and 11) with stable angina underwent primary stenting of a stenotic lesion in the left circumflex coronary artery and in the LAD, respectively. In 1 patient (patient 5) with unstable angina and 90% stenosis of the left main trunk, intracoronary stent implantation was performed because the patient refused to undergo coronary artery bypass grafting. In the 4 remaining patients (patients 6, 8, 9, and 10), stent implantation was performed for restenosis after PTCA. In these 11 patients, the interval between coronary stenting and death ranged from 2 days to 21 months.

A Palmaz-Schatz stent (Johnson & Johnson Interventional Systems) was inserted into 9 sites. One coronary artery received a Wiktor stent (Medtronic Inc); the remaining artery contained an ACS Multi-Link stent (Advanced Cardiovascular Systems).

All patients with coronary stents were treated initially with heparin and then maintained on different drug regimens: heparin only (patients 1, 2, 3, and 5); aspirin only (patient 11); aspirin and ticlopidine (patients 4 and 8); aspirin, warfarin, and dipyridamole (patient 9); and warfarin only (patients 6, 7, and 10). High-pressure

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stent dilatation, which recently became standard, was performed in 6 patients (patients 3, 6, 7, 8, 10, and 11).

Two patients (patients 7 and 8) developed recurrent angina pectoris, which was electrocardiographically related to the stented coronary artery. The signs and symptoms were considered indications of restenosis; angiography, however, could not be done in any of these patients.

**Histological Investigations**

In 9 of the 11 patients, the whole heart was available for study. The site of stenting was identified by comparing the clinical angiograms with the heart specimens. The coronary arteries were removed from the epicardial surface, and the site containing the stent was cut with scissors into 5-mm segments. Care was taken to have the articulation of the Palmaz-Schatz stent within 1 of these segments. In the present study, designed to investigate the cellular components at the stenting site with specific immunohistochemical techniques, we did not select an embedding method in methyl methacrylate recommended for obtaining intact cross sections. 7 In 4 hearts, the coronary artery segments were fixed in buffered formalin; in the remaining 5 hearts, the segments were fixed in methanol-Carnoy’s fixative.

After fixation, the stent fragments on the cut surface of each of the 5-mm segments were removed under a dissection microscope. Each 5-mm segment was then routinely processed and embedded in paraffin. From each segment, seconds were encountered that still contained residual stent fragments, these fragments were removed carefully, again by cutting the fragments and pulling them out of the paraffin-embedding block; thereafter, the sectioning continued. From each segment, ~100 to 300 sections were cut. This procedure thus allowed us to obtain an adequate overview of all the stented arterial segments.

In the remaining 2 patients (patients 5 and 9), only paraffin blocks from the sites of coronary stenting were available for the study; microscopic sections were made by use of the same method as described above. Of these sections, every 20th and 21st sections were stained with hematoxylin-eosin and Weigert’s elastic van Gieson’s stain, respectively. The other sections were used for immunohistochemical staining.

**Immunohistochemical Investigations**

**Single Staining**

The cellular components were analyzed by use of monoclonal antibodies against actin (1A4, HHF-35, and CGA-7), vimentin, macrophages (H-56 and PGM-1), and endothelial cell vWF. The 3 actin markers were used to study the state of differentiated SMCs. 1A4 and HHF-35 are markers that are the first to become positive when SMCs differentiate toward the contractile phenotype; once fully differentiated as such, CGA-7 also becomes positive. 

Sections were incubated with the primary antibody for 1 hour at room temperature. The sections were then subjected to a three-step staining procedure with the use of streptavidin-biotin complex with horseradish peroxidase for color detection. Buffered saline was used for washing between the subsequent incubation steps. Horseradish peroxidase activity was visualized with 3-amino-9-ethylcarbazole, and the sections were counterstained faintly with hematoxylin.

**Double Staining**

For the simultaneous identification of SMCs and macrophages, the following subsequent incubation steps were performed: normal goat serum (15 minutes), blotting, no washing, cocktail of 2 primary monoclonal antibodies (anti-SMC α-actin, 1A4 [IgG2a] and anti-macrophage. H-56 [IgM] for 60 minutes), cocktail of 2 secondary antibodies consisting of biotinated goat anti-mouse IgG2a and alkaline phosphatase–conjugated goat anti-mouse IgM (30 minutes), and β-galactosidase–labeled streptavidin (30 minutes). The enzymatic activity of β-galactosidase for 1A4 (IgG2a) was visualized in turquiose (BioGenex Kit, San Ramon), and that of alkaline phospho-
tase for HAM-56 (IgM) was visualized in red (New Fuchsin Kit, Dako). 11

Results

Palmaz-Schatz Stent (Nine Sites)

Three patients who had undergone emergency Palmaz-Schatz stenting after failed PTCA clinically developed subacute thrombosis 6 to 12 days after stenting. Gross investigation confirmed occlusive thrombus formation at the site of the stent (Figure 1). Light microscopical study showed that all sites contained a preexisting eccentric plaque and revealed that the struts of the stent had lacerated the arterial wall and caused a local response. At 6 days after stenting (patient 2), the site of the struts showed thrombus formation and an accumulation of macrophages around the struts. At 9 and 12 days after stenting (patients 3 and 4), the stent sites at the site of the thrombus revealed only minimal cellular reaction around the struts, whereas the stent sites proximal or distal to the thrombus showed a more prominent cellular reaction mixed with mural thrombus (Figure 2). In the latter, the cellular reaction was characterized by an early proliferation of neointimal tissue composed of abundant macrophages and actin-negative spindle-shaped cells, which stained positive for vimentin but negative with actin markers 1A4, HHF-35, and CGA-7 (Figure 2B through 2D). No positivity with the antibody against vWF was found at the luminal surface of the neointimal tissue (Figure 2E). Moreover, in these 3 patients, PTCA-related medial dissection at the border area between the plaque wall and plaque-free wall of a stented eccentric plaque was observed. The neointima alongside the dissection was also composed of macrophages and spindle-shaped cells, most of which were negative for the actin markers.

At 30 days after stenting (patient 5), the areas around the struts still contained remnants of thrombus, but there was distinct proliferation of α-actin–positive SMCs, with only occasional macrophages. No vWF-positive cells, covering the neointima, were observed at this site.

From 64 days on (patients 6, 8, 9, 10, and 11), all sites with stenting showed a distinct layer of neointimal tissue, albeit to varying degrees. In 4 lesions without clinical evidence of restenosis (patients 6, 9, 10, and 11; 64 days and 12, 15, and 21 months after stenting, respectively), the thickening of the neointimal tissue was distinctly less than in restenotic lesions (Figures 3 through 5; compare with Figure 6). In these nonrestenotic lesions, the neointima was composed predominantly of α-actin–positive SMCs, with some macrophages and foreign body giant cells located adjacent to the struts. The lesion at 64 days, moreover, showed fibrin, considered a remnant of a previous thrombus, within the neointima close to the luminal surface (Figure 3); no remnants of thrombus were found within the neointima at 12, 15, and 21 months after stenting (Figures 4 and 5). In the lesion at 64 days, positive staining with the anti-vWF antibody was observed partially at the luminal surface of the neointima. However, in lesions at 12, 15, and 21 months, a complete lining of vWF-positive endothelial cells was found at the luminal surface (Figure 5).

The site with clinical restenosis after stenting (patient 8; 5 months after stenting) presented exuberant neointimal formation, resulting in almost total occlusion (Figure 6). The neointima contained newly formed microvessels and scattered macrophages, particularly in the deep layers with extensive accumulation of SMCs. This patient had PTCA-related restenosis and thus had undergone stenting. The neointima formed after PTCA before stenting was clearly identified adjacent to the stent struts and distinct from the neointima induced by stenting. In fact, because of stent implantation, the post-PTCA neointima was markedly compressed, partially disrupted, and covered by a second neointima, which accounted for the post stent neointima (Figure 6).

Wiktor Stent (Patient 1, 1 Site)

The site 2 days after stenting showed occlusive intravascular thrombosis. Microscopically, PTCA-related medial dissec-
tion was present, and the stent struts had caused additional local laceration of the arterial wall.

**ACS Multi-Link Stent (Patient 7, 1 Site)**
The patient developed clinical signs and symptoms of restenosis and died 85 days after stenting. Gross and microscopic evaluations showed excessive neointimal formation, causing almost total occlusion (Figure 7). At the site of restenosis, the deep layers of the neointima contained abundant macrophages and newly formed capillaries, whereas the superficial layers consisted almost entirely of SMCs (Figure 7).

### Discussion
To the best of our knowledge, the present study, based on 11 stented sites in 11 patients and with an interval between stent implantation and death ranging from 2 days to 21 months, is the first systematic investigation into the pathobiology of stent stenosis in humans. Several aspects need further consideration. To this end, we will consider all 11 stents together, because the vast majority (9 of 11) were Palmaz-Schatz stents.

**Subacute Thrombosis**
Our series contains 4 patients (patients 1, 2, 3, and 4) in whom (sub)acute thrombosis after stenting had occurred. It is interesting that each of these 4 patients received stents because of a failure of a PTCA procedure that in each patient had led to a dissection extending into the media. Indeed, in patients in whom stenting is performed as a bailout procedure after PTCA failure, the reported incidence of thrombosis is relatively high and varies between

![Figure 2. Segment of the LAD of the same patient shown in Figure 1, 9 days after PTCA and stenting, which is taken more proximally and shows partial thrombotic adherence. A through E are serial sections. A, Hematoxylin-eosin stain. There is a distinct neointima (asterisks) with adherent mural thrombus at the site of a stent strut (arrowhead). B, Staining for vimentin reveals that the cells of the neointima are positive for vimentin. C, Anti–HHF-35. The spindle-shaped cells within the neointima do not stain with the α-actin marker at this stage (compare with B). D, Anti–HAM-56. The neointima contains HAM-56–positive macrophages. E, Anti–vWF. There are no positive-staining endothelial cells at the luminal surface of the neointima. There is positive staining of endothelial cells of the vasa vasorum (arrow), shown at higher magnification in the inset. Magnification A through D, ×110; E, ×72; inset E, ×217.](image)
6.9% and 16%. The question remains what mechanisms underlie the relationship between the initial PTCA-related medial dissection and the occurrence of subacute thrombosis after stenting.

Late Stent Stenosis
This study of human coronary arteries suggests that the sequence of events leading to a neointima formation after implantation of stents is basically similar to that encountered...
in animal models\(^{14-16}\) and is very similar to that observed in post-PTCA repair processes.\(^ {8-10,17}\) In general terms, the initial event is that of local thrombus formation, adjacent to the stent struts, gradually invaded by cellular components such as macrophages and \( \alpha \)-actin-negative spindle-shaped cells accompanied by the deposition of extracellular matrix components. This eventually differentiates into a much more fibrocellular lesion containing \( \alpha \)-actin-positive SMCs. These observations strongly suggest that mural thrombosis with macrophage infiltration at the earliest stage after stenting may be crucial in recruiting SMCs from the arterial wall. Indeed, platelet adherence and aggregation promote the subsequent healing process through the release of growth factors.\(^ {18-20}\) Macrophages also secrete a variety of cytokines and growth factors that have been shown in vitro to cause SMC proliferation and migration.\(^ {21-22}\) Experimental animal studies have suggested that PDGF, secreted not only from activated platelets but also from macrophages and other vascular cells, is 1 of the major growth factors involved in the process of neointimal formation after balloon injury.\(^ {23-25}\) Furthermore, our own in situ hybridization and immunohistochemical studies of human coronary arteries after PTCA using frozen sections have shown that PDGF-A and -B mRNAs, PDGF-B protein, and PDGF-\( \beta \) receptor protein are expressed in neointimal lesions containing macrophages and actin-negative spindle-shaped cells.\(^ {10,26}\) Whether similar phenomena occur in the process of neointimal formation in human coronary arteries after stenting is unknown, but the possibility is intriguing, and future studies using frozen sections obtained from stenting sites should look into these aspects.

In 1987 in their study of coronary arteries of dogs, Schatz and coworkers\(^ {16}\) considered intimal hyperplasia a consequence of intense fibroblast proliferation at 8 weeks after stenting. The present study in humans, using immunocytochemical markers, clearly shows that cellular components invading the thrombotic aggregates at the sites of laceration at the earliest stages are spindle-shaped cells negative for actin markers such as HHF-35 and 1A4. However, at 30 days, a neointima is clearly identifiable, and the spindle-shaped cells present within this layer appear to be positive with both actin markers. This sequence strongly suggests that during the evolution of the neointima, the \( \alpha \)-actin-negative spindle-shaped cells most likely represent dedifferentiated SMCs, which in time gradually redifferentiate into \( \alpha \)-actin-positive SMCs. This concept of staged redifferentiation of neointimal SMCs is endorsed by experimental studies\(^ {27-29}\) and is relevant because we previously demonstrated almost identical shifts in the cytoskeletal phenotype of spindle-shaped cells during the

Figure 5. Segment of the LAD 21 months after stenting (patient 11 in the Table). A, Gross aspect of a cross section shows an eccentric atherosclerotic plaque (AS) and extensive neointimal formation with considerable luminal narrowing. The stent struts are clearly visible within the neointima. B, Elastic tissue stain of the section shown in A. There is extensive neointimal tissue (asterisks) around the stent struts (arrowheads). C, Same section as shown in B immunostained with 1A4. The neointima around the stent struts (arrowheads) is composed almost totally of SMCs. D, Staining for vWF. The luminal surface of the neointima is lined by vWF-positive cells, considered regenerated endothelial cells. Magnification B and C, \( \times 33; \) D, \( \times 368.\)
development of the neointima after angioplasty injury in human coronary arteries. It could be argued that the α-actin–negative spindle-shaped cells observed early in the neointima might represent not only dedifferentiated SMCs but also other cell types such as infiltrating macrophages that have lost their specific antigen or fibroblasts—a point of view that at this stage, cannot be elucidated.

Despite similarities between experimental animal models and humans, the present study also shows important differences in the healing phenomena after stenting. First, a restenotic lesion, which occurred 85 days after implantation of the ACS Multi-Link stent (patient 7) and 5 months after implantation of a Palmaz-Schatz stent (patient 8), showed excessive neointimal proliferation composed of numerous macrophages; SMCs; and abundant, newly formed microvessels, which led to almost total occlusion of the lumen (Figures 6 and 7). It is the quantity of these features that makes them exceptional; neovascularization and macrophages with occasional giant cells are common findings, particularly adjacent to stent wires, in both experimental animal models and this study in human coronary arteries. These observations are of interest because both sites of restenosis after stent implantation show large numbers of macrophages and extensive neovascularization, features not observed to this extent at “nonrestenotic” stent sites. In this context, it is important that Moreno and associates recently showed that the amount of macrophages in preexistent atherosclerotic lesions is a predictor for restenosis after PTCA. Previously, Strauss et al
compared coronary atherectomy material obtained from patients with restenosis after stenting with that obtained from patients with restenosis after angioplasty or atherectomy. They concluded that SMC proliferation was the predominant feature in restenotic tissue regardless of the initiating procedure and without any unique features attributable to stenting in general or to a particular type of stent. This apparent controversy is of considerable interest. We have indicated previously that once the post-PTCA laceration, particularly in concentric lesions, is limited to the atheroma, the repair...
process is often accompanied by neovascularization and accumulation of macrophages in the deeper parts of the neointima.10,17,26 Hence, the formation of a concentric layer of neovascularization and macrophage accumulation in the deep parts of the neointima may result from organization processes of thrombosis, which initially was formed around the stent wires at the sites of initial laceration. Accumulation of SMCs at the luminal side follows at a later stage.

Another difference with the earlier experiments by Schatz and coworkers18 performed on normal coronary arteries in dogs is that in their series, the maximal neointimal thickness occurred at 8 weeks after stent implantation; this was followed by sclerotic changes with less cellularity and marked regression of the neointima by 32 weeks.16 However, in our series of human coronary arteries, each containing atherosclerotic disease, we observed lesions with intervals between stent implantation and death ranging from 12 to 21 months, and each showed distinct cellularity composed predominantly of α-actin–positive SMCs without obvious sclerotic changes. These observations clearly indicate that fibrocellular as seen in normal dog arteries certainly cannot be considered the rule in human atherosclerotic coronary arteries, at least not within the limits of our study, which is 2 years after stenting. It remains to be settled in future studies with more cases whether the differences between our observations and those obtained in dogs relate merely to species differences or to the fact that normal arteries in dogs cannot be considered to represent the basic mechanisms that underlie poststentering neointimal formation in atherosclerotic lesions in humans.

In the experimental dog model used by Schatz and coworkers,16 reendothelialization after stenting, using the scanning electron microscope, appeared incomplete at 1 week. At 3 weeks, a mosaic type of endothelial cell lining was found, and full endothelial covering with mature and elongated cells was seen at 32 weeks.16 Thus far, 1 autopsied case, 8 weeks after a Palmaz-Schatz stent implantation, also reports a neointima covered by endothelial cells overlying the area of the stent.17 However, there is no immunohistochemical identification of these endothelial cells. In the present study, with regard to immunostaining characteristics with anti-vWF antibody, non-restenotic lesions after stenting revealed partial endothelial cell regeneration at 64 days and complete restoration of the endothelial cell lining in lesions at 12, 15, and 21 months after stenting. Further studies with more cases are needed to clarify a more detailed time course of reendothelialization at the site of coronary stenting in humans. Nevertheless, the present study, for the first time, provides immunohistochemical data regarding reendothelialization after stenting in human coronary arteries.

Restenosis Versus Nonrestenosis

Previous studies have suggested that chronic stent recoil may contribute significantly to stent restenosis.21,32 However, recent serial intravascular ultrasound studies have demonstrated that chronic stent recoil is minimal and that late luminal loss and in-stent restenosis are the result of neointimal tissue proliferation.33–35 In the present study, neointimal proliferation was present from 9 days after stenting on. The salient histological features were basically the same whether restenosis had developed or not. Indeed, from this small series, it appears that the differences between restenosis and “nonrestenosis” are quantitative rather than qualitative. Be that as it may, the observations presented here strongly support the concept that stent stenosis is caused by neointimal proliferation.

Study Limitations

This study represents only a limited number of autopsied cases obtained from patients after implantation of a coronary artery stent, most of which were Palmaz-Schatz stents. We presented only 1 case with a Wiktor stent and 1 with an ACS Multi-Link stent. The question arises, therefore, whether the findings obtained at the site of stenting with the Palmaz-Schatz stent apply to other stents; the observations suggest that this is so, but the limited number of cases does not allow a firm statement to this end.

References


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