CORONARY STENTING AS AN ADJUNCT TO BALLOON ANGIOPLASTY

CORONAIR STENTING ALS AANVULLING OP BALLON DILATATIE

Proefschrift

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Cover: Restenosis 6 months after implantation of a stent in a bypass graft. In the follow-up angiogram (upper photo) the outline of the stent appears slightly radiopaque and hyperplasia has resulted in a complex narrowing within the vessel segment containing the stent. In the gross specimen (lower photo) of the surgically retrieved bypass graft containing the segment shown in the upper photo, the longitudinal cross-section of the vessel shows the stent filaments protruding from the wall. The striking similarity between the angiographic contours of the vessel and its actual appearance is evident.

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Aan Terry,

en onze lieve dochtertjes Orlee-Rose en Shira

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Chapter 1

Introduction

Introduction and Overview of this Thesis

Andreas Gruentzig initiated the era of interventional cardiology in 1977 with the introduction of percutaneous transluminal coronary angioplasty (PTCA) (1). The acceptance of coronary angioplasty is obvious by the widespread use of the procedure (>300,000 cases in the United States in 1990), the growing list of patient indications and the existence of an extensive infrastructure to support the procedure (including the expansion of catheterization laboratories, fellowship trainee programmes, and substantial involvement by industry that has dedicated itself to continued improvement in advanced x-ray imaging systems, and angioplasty hardware equipment). Yet despite the growth and success of PTCA, the first human coronary stent implantations were performed in 1986 in Lausanne, Switzerland by Ulrich Sigwart and in Toulouse, France by Jacques Puel (2). In fact, in the past five years several other devices have been clinically tested as alternatives or adjuncts to PTCA including directional atherectomy, rotational abrasion, and different types of laser therapy. These devices have been introduced due to the limitations of balloon angioplasty, namely early occlusion and restenosis within the first 6 months following angioplasty, which have persisted despite extensive attempts to find pharmacological solutions to these problems (3-5).

Stents are devices that are implanted intraluminally to provide mechanical support. A number of devices have been developed that differ in composition, design, and mechanical behavior. According to Dr. Philip Urban of Geneva, the initial attempts to implant tubes inside vessels, are described in an article published by Alexis Carrel from New York in September 1912 in the American monthly "Surgery, Gynaecology and Obstetrics" (6). In these early experiments, 7 dogs were implanted with short glass tubes into the thoracic aorta, 3 animals received aluminium tubes and in 1 animal a gold plated alluminium tube was inserted through a surgical incision inside the thoracic aorta. Thrombosis of the tube occurred in 5 cases between 5 and 97 days after the implantation. Two animals died from hemorrhage 8 and 11 days following the operation.

In 1969, Charles Dotter revived the concept when he implanted impervious plastic tube grafts in the normal canine femoral and popliteal arteries (7). His initial attempts failed due to thrombosis within the first 24 hours but patency rates improved when he substituted stainless steel coils instead of tubes and included heparin infusions for 4 days after implantation (8). In 1991, a variety of endovascular stent designs are under clinical and experimental evaluation. Changes in design, the use of metal alloys and miniaturization of the endoprostheses has resulted in the availability of several type of stents for experimental and clinical evaluation.

The central topics of this thesis are the coronary stent and the response of the vessel wall after implantation. In chapter 2, as a prelude to the quantitative angiographic analyses of the coronary stent, the rationale for stenting an atherosclerotic stenosis as an adjunct to balloon angioplasty and the hyperplastic processes triggered in the vessel wall as a result of combined balloon dilatation and stent implantation are examined, based on our animal studies and human saphenous vein bypass grafts that

were surgically removed 3 days to 10 months after stenting.

In chapters 3 and 4, some methodologic aspects of performing quantitative coronary angiographic (QCA) measurements in stented vessels are presented. The optimal method to analyse the immediate and late angiographic results after PTCA or stenting has not been determined. Although edge detection remains the main form of analysis, its use may be limited in eccentric lesions, particularly after dissections disrupt the anatomy of the vessel. Densitometry has been proposed as an alternative method because it theoretically is independent of the geometric shape. In chapter 3, we performed an in-vitro study to assess the contribution of 3 currently investigated coronary stents to the densitometric measurement of a known stenosis contained within plexiglass phantoms. The results showed that stainless steel stents (the Wallstent and the Palmaz-Schatz stent) cause only minor increases (<8%) in the calculated minimal luminal cross-sectional area. However, tantalum-containing stents (eg. the Wiktor stent) may result in serious overestimation of lesion area by densitometry derived measurements, particularly if contrast is diluted or the vessel is not well filled. In chapter 4, we compared the results of minimal luminal cross-sectional area obtained by edge detection versus densitometry in 19 patients who underwent coronary stenting with the Wallstent. Although poor correlations between these two methods has been observed in previous studies after PTCA, we observed a much closer correlation after stenting, probably because of the smoothing of the vessel contours by the stent and remodeling of the stented segment into a more circular configuration.

In chapters 5-8, the early and late follow-up results of the first clinically implanted coronary stent, the Wallstent, are reviewed. The six European centers involved with testing this device agreed to set up an angiographic core laboratory at the Thoraxcenter in Rotterdam for quantitative angiographic analysis to ensure objective assessment of the results. The Coronary Artery Analysis System (CAAS) which has been extensively validated, was used for all quantitative studies. In addition to the angiographic analysis, brief clinical histories were available. Clinical follow-up was obtained from patient charts and personal queries to the participating cardiologists. In chapter 5, the results of the initial 117 self-expanding stents implanted in 105 patients are presented. This study represents the first quantitative follow-up study of one of the new post angioplasty devices and is therefore of historical importance. The main feature of this report is the high occlusion rate (24% overall) which appeared to be due in part to patient selection, differing and changing anticoagulation regimens at the various centers and operator inexperience. Restenosis was also documented in 14% of the patent stents at follow-up, which in the absence of the occlusion problem, would compare favorably to historical PTCA studies.

Chapters 6 and 7 include data collected on the 265 patients who had stent implantations performed in 308 lesions from March 1986- March 1990. In chapter 6, we have identified the angiographic predictors of restenosis within the coronary Wallstent using a relative risk analysis. The use of multiple stents/lesion, oversizing the stent (unconstrained stent diameter exceeding reference diameter > 0.7 mm), bypass grafts and residual diameter stenosis significantly increased the risk of restenosis using either of two restenosis criteria. Since some of these factors can be avoided (excessive oversizing, use

of multiple stents, more optimal initial dilatation), recommendations to lessen the risk of restenosis in stented vessels are suggested. In chapter 7, an in-depth analysis of clinical results, occlusion rates and restenosis rates is presented for the entire study group and then according to date of implantation and vessel type. Since our experience with stenting was the first of its kind, the indications for and management of patients implanted with this particular prosthesis evolved with increased experience and knowledge. Coronary artery disease in the early patients (partly described in chapter 5) consisted predominantly of native vessels that had previously restenosed. The later patients mainly consisted of patients with bypass grafts who were stented for primary de-novo coronary lesions and native vessels stented as part of a bail-out strategy following complicated balloon angioplasty. Although not statitstically significant, there was a reduction in the in-hospital occlusion rate to 12% in the later group accompanied by an increase in the restenosis rate from 21% to 32%. The palliative role of stenting was also evident in the low actuarial event-free survival (freedom from death, myocardial infarction, bypass surgery or PTCA) for native vessels (46% at 40 months) and for bypass grafts (37% at 20 months).

In chapter 8, the separate Thoraxcenter experience of stent implantations in diseased bypass grafts is presented in detail. Stenting is discussed as a treatment option in this growing group of patients that frequently do not respond well to reoperation or conventional balloon angioplasty. All of the patients stented at the Thoraxcenter had severe symptoms and the majority were considered high risk for surgery or PTCA. In hospital occlusion was noted in 10% of the patients and usually occurred in the setting of acute ischemic syndromes or anticoagulation interruption. Although stent related restenosis was high (47%) and patients frequently required reintervention, coronary stenting can be effective therapy in some medically refractory patients who are poor candidates for alternative procedures.

In chapters 9-11, the results of examining coronary tissue removed by directional atherectomy from vessels including restenosis within coronary stents, by a number of histological, and molecular biology techniques are described. In chapter 9, the early and late results of directional atherectomy performed for restenosis within the coronary stent of 9 patients (10 procedures) are examined. In addition, the restenosis tissue was studied for cell identification, proliferation rates and cell density using a combination of light and electron microscopy and immunohistochemistry techniques. The results were compared with a control (non-stented) group of patients who had restenosis tissue removed 14-597 days following an initial procedure (PTCA, atherectomy or laser). The angiographic results showed that directional atherectomy is an effective form of treatment of restenosis within the stent but is also limited by recurrences. The tissue studies indicated that smooth muscle cells are the main cell type found in restenosis lesions regardless of the initial type of procedure and that smooth muscle cell proliferation is an early event and barely detectable 2 months after the procedure. In addition, the vast majority of smooth muscle cells modulate towards the contractile phenotype early after the procedure and the lesion cellularity decreases as a function of time but with a large interindividual variability.

In chapter 10, the origin of the smooth muscle cells found in coronary lesions was shown in a de novo human coronary artery stenosis after heart transplantation. A technique (DNA fingerprinting) was

used that is based on the differences in genetic content between donor and recipient at several highly polymorphic variable number of tandem repeat (VNTR) gene loci. These studies were performed on DNA that was isolated from the coronary atherectomy specimen along with samples from the donor myocardium and the recipient's blood, after amplification of the DNA by the polymerase chain reaction (PCR). The electrophoresis results confirmed that the vascular intimal lesions typically seen in atherosclerosis and restenosis consist primarily of smooth muscle cells that are derived from within the vessel wall and not the circulation.

In chapter 11, the preliminary results of culturing smooth muscle cells from coronary atherectomy specimens (including restenotic lesions in stented areteries) are presented and compared with smooth muscle cells isolated from the media of umbilical arteries. The main findings of this study were that smooth muscle cells can be serially passaged from a minority of lesions and that these cells show lower proliferation rates (50%) but higher rates of synthesis of the extracellular matrix components, collagen (50%) and sulfated glycosaminoglycans (>2x) than the umbilical smooth muscle cells. This cell culture system appears to be a useful model to study the processes of atherosclerosis and restenosis.

The goals of this thesis are twofold. Interventional cardiology has now entered the post balloon angioplasty period and we are faced with challenges unforseen 15 years ago. We have witnessed an explosion of alternative devices to balloon angioplasty such as several types of stents, at least three different atherectomy devices and a cornocupia of different laser catheters (energies, designs etc). Clinical acceptance of any of these new techniques must await rigorous scientific evaluation. At the present time, this can only be accomplished with the use of quantitative coronary arteriography. In this thesis, we have attempted to set a standard for objective angiographic analysis and reporting. Although other methods of evaluation may be implemented in the future (for example, intravascular ultrasound), interventional cardiologists must not restrict themselves to the technical aspects of some of these more demanding and complicated devices, but must be active in the acquisition of new knowledge that increases our understanding of the processes such as restenosis that hamper the late outcome. We must devote our efforts to combine research in the basic mechanisms of the biologic processes that we initiate with our interventions, with a rigourous scientific evaluation of our efforts in the catheterization laboratory. I hope that the studies described within this thesis will be considered with these goals in mind.

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Chapter 2

Stenting of coronary arteries. Has a modern Pandora's Box been opened?

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NEW TECHNOLOGY

Stenting of Coronary Arteries: Has a Modern Pandora's Box Been Opened?

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Interventional cardiology has recently witnessed the growth of several alternatives to percutaneous transluminal angioplasty, including coronary stenting. Although stenting appears to be useful in treating abrupt closure after coronary angioplasty, its effectiveness in limiting the complex processes responsible for late restenosis is much less certain. Pathologic examination of stented human saphenous bypass grafts shows extensive deposits of platelets, fibrin and leukocytes along the stent wires within the 1st week and formation of a neointima of variable thickness after 3 months without evidence of foreign body reaction. The long-term effects of continuous barotrauma induced by the expanded stent remain unknown. It is difficult to assess the relative merits of the new devices, but stenting has several theoretic advantages. It seems less disruptive to the underlying architecture of the vessel wall and enjoys favorable theoretic and effective expansion ratios. Widespread clinical acceptance for stenting will depend on demonstrating that its safety, efficacy and cost efficiency are superior to those of balloon angioplasty.

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The introduction of coronary balloon angioplasty by Andreas Gruentzig (1) in 1977 provided the stimulus for rapid technologic growth in the field of interventional cardiology. This development has produced several new devices designed to ablate coronary artery narrowings, recanalize occluded vessels and prevent restenosis. It is difficult to evaluate the relative merits of each intervention and to define their roles in clinical cardiology. In applying this new technology, cardiologists have limited their concern to the technical and procedural aspects, while sometimes overlooking the complex biologic and physiologic mechanisms of atherosclerosis, and in particular of the restenosis process. In achieving the perceived benefit of therapeutic intervention with these devices, the vessel wall is subjected to thermal and mechanical insults that may have hidden long-term consequences. An example is the restenosis process, which has been iatrogenically induced in tens of thousands of patients.

One of these newer developments has been the use of endoluminal vascular prostheses, although the original concept of intravascular stenting precedes the introduction of coronary artery interventional cardiology by many years. Since the original description of Dotter's tubular coil spring stent (2), many variants of the original concept have been introduced, including thermal shape memory alloy stents (3–6), steel spirals (7), stainless steel mesh stents (8–11), slotted stainless steel tubes (12–15), zigzag stents (16–18), U-configuration bends (19), interdigitating coils (20–22), tantalum helical coil stents (23), knitted tantalum wire stents (24,25), removable, metallic mesh stents (26) and synthetic polymeric and biodegradable stents (27). These various devices differ greatly in their fundamental geometry (tube, mesh or single wire), composition (metal or plastic) and mechanical behavior (active or passive expansion). Furthermore, there are a variety of subtle differences that may be important in themselves, such as thickness of filaments, alloy composition, electrostatic behavior and biocompatible or therapeutic coatings.

More than 7 years have passed since the first clinical report (8) of successful coronary stent implantations. Although the current world experience has now exceeded 1,000 stent implantations, the clinical indications and applications of this prosthesis remain undetermined and even experienced investigators are uncertain on these issues. The current status of the coronary stent parallels that of other recently introduced technologic advances, including laser angioplasty and atherectomy (directional and nondirectional types). This raises a fundamental question: have we been unable to realize the full potential of these newer devices because of our limited understanding of the underlying biologic interactions, particularly those responsible for restenosis? In this review, we address several relevant issues based on our experience in the evaluation of various coronary stents.

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Rationale for Stenting an Atherosclerotic Stenosis During or After Balloon Dilation

Short-term consideration: abrupt closure. Abrupt vessel closure after angioplasty occurs in 2% to 11% of procedures (28–31). Intimal flaps induced by the arterial injury can disrupt flow by partially or completely occluding the lumen. Sluggish anterograde flow and the exposure of media to procoagulant blood-borne elements are potent thrombogenic stimuli that further contribute to the process. The coronary stent, by acting as a splint, can physically contain the protruding obstructive flap and maintain flow as well as possibly prevent distal embolization of macroscopic debris originating from the plaque or flap (32). This scaffolding function appears to be a property common to both balloon and self-expanding stenting prostheses. Angiographic studies (10) after stent implantation have shown that the self-expanding Wallstent has a smoothing effect that reduces calculated poiseuille and turbulent contributions to flow resistance. Several reports have documented successful deployment of the various types of stents in the "bail-out" situation when the presence of intimal dissection has led to a poor and even critical hemodynamic result (33,34).

Long-term considerations: restenosis. Restenosis remains the major limitation of coronary angioplasty. Despite a lack of uniformity of definition, several angiographic follow-up studies have documented a 20% to 40% incidence in the 1st 6 months after angioplasty (35–38). Restenosis has been defined angiographically as a significant deterioration in the luminal diameter of a lesion that had previously been successfully dilated, and does not necessarily indicate a common pathologic substrate. Diverse histologic processes may be responsible for restenosis depending on the time interval since angioplasty.

Early restenosis. Restenosis has been documented in up to 11% of lesions as early as 1 to 4 days after coronary angioplasty (38–40). It is believed that the early cases of angiographic worsening are a result of several processes, including elastic recoil, vasospasm or platelet-fibrin thrombi, or combinations. This time interval is too brief for significant fibrointimal hyperplasia to have occurred, for several reasons. Pathologic studies (41–43) of vessels retrieved <10 days after angioplasty have not shown any significant intimal hyperplasia. Animal experiments (44,45) in carotid arteries of rats have demonstrated that smooth muscle cell migration into the intima begins only at 4 days and maximal intimal smooth muscle cell proliferation is not noted before 7 days after balloon endothelial denudation. Furthermore, cell cultures of medial smooth muscle cells (46) have shown that the modulation of phenotype from the quiescent, contractile state (typical of normal medial smooth muscle cells) to a metabolically active, synthetic state occurs only after 6 to 7 days. Smooth muscle cells obtained from intimal thickenings phenotypically resemble these synthetic-type smooth muscle cells observed in culture and share a common cytoskeleton protein profile that differs from typical medial smooth muscle cells (47,48).

The stent and elastic recoil. The significance of elastic recoil has been demonstrated acutely during angioplasty. In a study of 151 dilated segments, the minimal luminal cross-sectional area before angioplasty was 1.1 ± 0.9 mm^2 (49). Immediately after the procedure the cross-sectional area of the dilated vessel was 2.8 ± 1.4 mm^2. Elastic recoil, defined as the difference between the balloon cross-sectional area (5.2 ± 1.6 mm^2) and the vessel area after angioplasty, was calculated to be 2.4 ± 1.4 mm^2, which is almost 50% of the cross-sectional area of the fully inflated balloon. In an angiographic study of the initial 117 stent implants, we demonstrated that the self-expanding Wallstent mitigates the effects of elastic recoil. Stenting immediately improved the minimal cross-sectional area from 3.0 ± 1.2 mm^2 after angioplasty to 5.5 ± 2.7 mm^2. In a subgroup of patients with angiography 24 hours later, the stent continued to expand and increased the cross-sectional area to 6.8 ± 4.4 mm^2 (Serruys et al., unpublished observations).

Late restenosis. Two processes have been implicated in the development of late restenosis. In some cases it has been attributed to the organization and fibrous conversion of platelet-fibrin thrombi that form at the site of intimal damage. However, a more important mechanism appears to be marked cellular proliferation within the vessel wall that is stimulated by complex interactions between platelets adherent to the damaged intima, macrophages, endothelial cells and medial smooth muscle cells. Pathologically, late restenosis is characterized by an aggressive proliferation of smooth muscle cells that presumably have migrated from the media into the intima, resulting in a variable degree of luminal narrowing (43,50). Immunoperoxidase staining of the cellular component of this fibrointimal tissue has identified the characteristic cytoskeleton proteins of medial smooth muscle cells—alpha actin, desmin and vimentin—confirming the origin of the cells responsible for this growth.

What Causes Smooth Muscle Cell Proliferation?

Abnormal smooth muscle cell proliferation is an intricate process that is only partially understood. Animal models have revealed that balloon denudation in arteries will stimulate a sequence of events if either of two conditions is present: 1) extensive endothelial denudation, or 2) significant medial smooth muscle cell injury.

The pioneering work of Reidy et al. (44,45) showed that significant intimal hyperplasia occurred after balloon injury in rat carotid arteries that resulted in the loss of up to 25% of the vessel wall deoxyribonucleic acid (DNA). This loss reflects widespread medial smooth muscle cell injury, since endothelial cell loss alone could not account for such a major change in DNA content. Later, more sophisticated techniques of vessel wall injury were used (51), which localized

damage to the endothelium, sparing the subendothelium and medial layers. Subsequently, intimal thickenings developed only in regions of the vessels that were not re-covered with endothelium after 7 days. These studies suggested that smooth muscle cell proliferation and migration are separately controlled processes, because some areas of rapid endothelial regrowth contained increased numbers of medial smooth muscle cells without a corresponding increase in intimal thickness. A separate series of autoradiographic experiments (52,53) showed that only 50% of intimal smooth muscle cells are capable of proliferation, supporting this concept.

Platelet-derived growth factor. Several mitogens have been implicated in the stimulation of smooth muscle cells. Platelet-derived growth factor, the most intensively studied factor, is a dimer compound composed of two homologous polypeptide chains (A and B) that are disulfide bonded (54). Although platelet-derived growth factor was originally isolated from platelets, further study has confirmed that it is released from several different cells, including vascular endothelium, macrophages and even activated smooth muscle cells, perhaps explaining why smooth muscle cells continue to proliferate long after the initial platelet-vessel wall interaction (55–58).

The binding of platelet-derived growth factor to its receptor initiates a complex cascade of signal transduction within the cytoplasm and ultimately into the nucleus of the smooth muscle cell, resulting in cell division and protein synthesis. Although these pathways have not been elucidated fully, important steps include the platelet-derived growth factor receptor-mediated phosphorylation of tyrosine kinase and activation of phospholipase C, which subsequently generates two important second messengers, diacylglycerol and inositol triphosphate (59,60). The platelet-derived growth factor receptor and both its chains have been sequenced and it is now possible to clone platelet-derived growth factor with recombinant technology. Monoclonal antibodies against both chains of platelet-derived growth factor and its receptor have also been produced. The gene that codes for the B chain mRNA of platelet-derived growth factor is c-sis, which is the cellular counterpart to the v-sis gene of the simian sarcoma virus. An intriguing connection between neoplasia and atherosclerotic lesions is the demonstration of an active human oncogene in atherosclerotic plaques (61). Cultured mouse fibroblast NIH 3T3 cells have been transformed with transfected DNA from these plaques (61). These transformed cells have established slow-growing tumors in "nude" mice.

Other growth factors. Other important growth factors that have been related to restenosis include interleukin-1 (IL-1), fibroblast growth factor, colony stimulating factor, epidermal growth factor, insulin-like growth factor (somatomedins), endothelin and serotonin. The relative influence of the individual factors and possible interactions are largely unknown and indicate our limited understanding of the entire process.

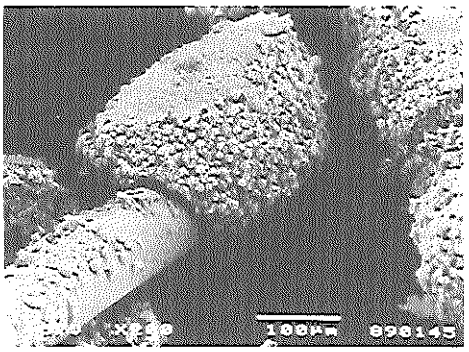


Figure 1. Scanning electron micrograph of a stented human saphenous vein bypass graft removed 3 days after implantation. Extensive deposits of platelets, leukocytes and fibrin are evident on this detail of the stent filaments.

Pathology of Restenosis: After Coronary Angioplasty and Coronary Stenting

Although autopsy reports of late follow-up angioplasty cases (3 to 20 months) are limited, the general consensus is that the characteristic features of restenosis are smooth muscle cell hyperplasia and a variable amount of extracellular matrix and fibrosis depending on the time elapsed since angioplasty. In addition to data from chronic animal studies, stented venous bypass grafts have been retrieved from several patients for analysis. Although the extent of intimal hyperplasia is similar after stenting to postangioplasty examination, several histologic features appeared to be unique to coronary stents.

Pathologic and histologic features. In human saphenous vein bypass grafts and porcine coronary arteries retrieved 3 to 7 days after stent implantation, extensive deposits of platelets, fibrin and leukocytes are observed along the stent wires (Fig. 1). In the pig, the stent wires become embedded in the vessel wall and are covered with a neointima within 7 days. This neointima consists of organizing thrombus directly adjacent to the wire and several layers of smooth muscle cells along the luminal surface (Fig. 2). Scanning electron microscopy has confirmed complete endothelialization.

At 4 weeks in porcine coronary arteries, few traces remain of the initial platelet-fibrin thrombus, which is represented by a few erythrocytes, leukocytes and lipid-laden foam cells that are interspersed in a disorganized fibrocellular layer (Fig. 3). At the luminal side two distinct layers of smooth muscle cells are present, one in a circular orientation immediately below the endothelium and a deeper layer in a longitudinal orientation.

After 3 months, a more extensive neointima forms in the

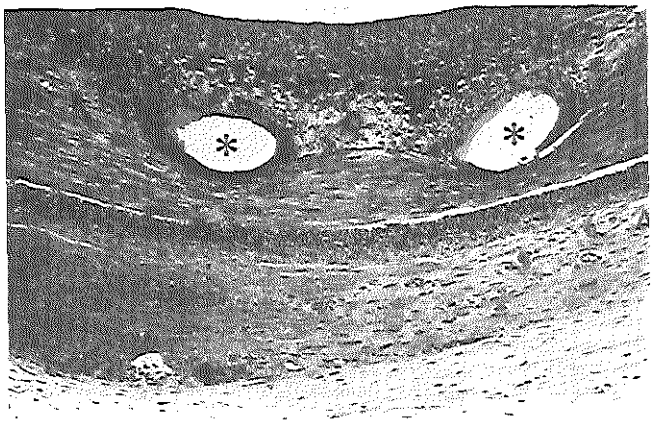
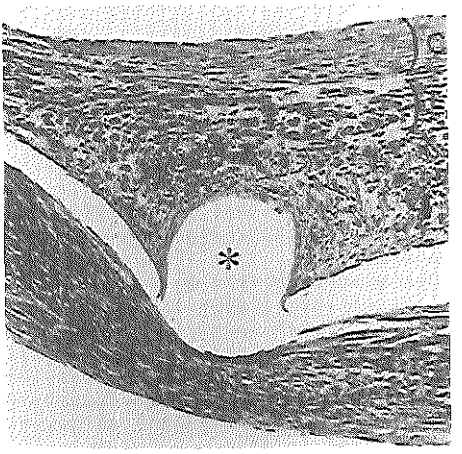


Figure 2. Light micrograph of a stented porcine femoral artery 7 days after implantation. The voids (*) represent the sites of the 70 μm diameter stent wires, which have been removed. There is a disorganized layer of neointima (N) on the luminal aspect of the stent wire, containing smooth muscle cells, trapped red blood cells and fibrin. Above the disorganized layer is an organized neointima that contains smooth muscle cells covered by endothelium. The internal elastic lamina is interrupted at the left (**arrow**). A = adventitia; M = media.

porcine coronary artery with only a small area adjacent to the stent wire containing leukocytes and cellular debris (the so-called "Bermuda triangle") (Fig. 4). In human saphenous vein bypass grafts removed 3 to 10 months after the stenting procedure, the amount of neointima which develops is comparable with the amount of neointima in the porcine coronary artery, but the neointima in humans borders on the old atherosclerotic plaque (Fig. 5). At the junction between old plaque and recent neointima, abundant foam cells and extracellular lipid deposits are found within the new neointima in addition to extensive extracellular matrix production (Fig. 6).

Mechanisms. The causes and possible relations between these early and late histologic features are unknown. Two factors may be important. First, the regenerated endothelium that covers the stented segment may be dysfunctional and thus permit abnormal and excessive lipid infiltration and macrophage penetration across the endothelial barrier. Scanning electron microscopy of the endothelial lining has indicated an irregular, raised endothelial surface in lieu of the normal smooth covering (Fig. 7) although no permeability to Evan's blue dye was demonstrated in stented porcine arteries after 3 months (Van der Giessen et al., unpublished observations). Second, important chemotactic substances may be released by the cellular debris trapped in the tissue adjacent to the stent wires. This area appears to persist late after stenting for several reasons, including continued damage from direct pressure necrosis and its deeper location in the vessel wall, which isolates it from laminar flow patterns predominating on the luminal aspect of the stent wires. Striking similarities exist between the biology of stented vessels at 3 months and chronic atherosclerotic lesions, namely, proliferation of smooth muscle cells, large amounts of connective tissue matrix including collagen, elastin and proteoglycans, and lipid accumulation in the form of foam cells (smooth muscle cells and macrophages), and extracellular deposits. The natural history of these post-stent lesions has not yet been determined.

Figure 3. Light micrograph of a porcine coronary artery 4 weeks after implantation, showing the remnants of the initial thrombus, which now contains a few erythrocytes, leukocytes and lipid-laden foam cells that are interspersed in a disorganized fibrocellular layer. At the luminal side, two distinct layers of smooth muscle cells are present, one in a circular orientation (C) immediately below the endothelium, and the other a deeper layer in a longitudinal orientation (L). (*) = void representing the site of a removed 127 μm diameter stent wire.



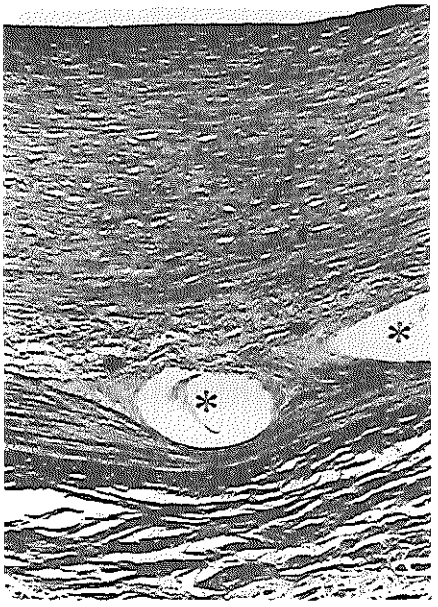


Figure 4. Light micrograph of a stented porcine artery 3 months after implantation. A more extensive neointima has formed, with only two small areas adjacent to the stent wire containing leukocytes and cellular debris (arrows). (*) = void representing the site of a removed 70 μm diameter stent wire.

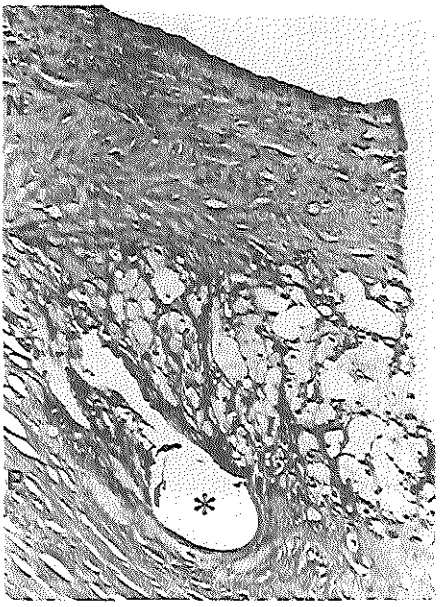


Figure 5. Light micrograph of a human saphenous vein bypass graft removed 10 months after stent implantation. A prominent neointima (N) has formed and borders on the old atherosclerotic plaque (P). At the junction between the old plaque and the recent neointima, abundant foam cells are found in the new neointima. (*) = void representing the site of a removed 70 μm diameter stent wire.

Stenting and Hyperplasia

Role of stenting in hyperplasia. Several possible theories have been advanced to support the role of stenting in limiting intimal hyperplasia (32,63,64). However, there is minimal experimental evidence to justify this position, and available animal and clinical studies confirm that significant hyperplasia occurs within the stented segment. The extent and characteristics of this hyperplasia are illustrated by the case of a 67 year old man who had recurrence of angina 3 months after stenting in a bypass graft. Angiography revealed a severe narrowing within the stent that was treated by combined balloon angioplasty and atherectomy (Fig. 8). The tissue specimen removed by the atherectomy device is shown in Figure 9. The microscopic evaluation shows abundant extracellular collagenous matrix and areas of marked cellularity that stain positively for two smooth muscle cell cytoskeleton proteins, alpha actin and vimentin (Fig. 10).

Although hyperplasia is a consequence of stenting, the functional significance of this growth may be diminished. This is explained by the intrinsic dilating property of the self-expanding stent, which initially improves luminal area 50% more than angioplasty by itself and in many patients more than compensates for the late proliferation. Unfortunately, the ideal ratio of stent size to vessel size that will result in optimal dilation with minimal compensatory hyperplasia remains unknown. The importance of this relation to the final outcome was illustrated recently by a Mayo Clinic study (65) in which a model for restenosis was developed by implanting stainless steel and tantalum coils with markedly oversized angioplasty balloons inflated to high pressures up to 14 atm.

Confounding Aspects of Stenting

There are three additional aspects of stenting that further confound our understanding of the processes occurring in the vessel wall after injury.

1. Foreign body interactions with the vessel wall. In contrast to brief, transient balloon-induced injury, nonbiodegradable stents are permanent foreign bodies with potentially important interactions due to type of metal, electrostatic charges, and possible physical irritation from individual filaments. Whether the continued presence of a

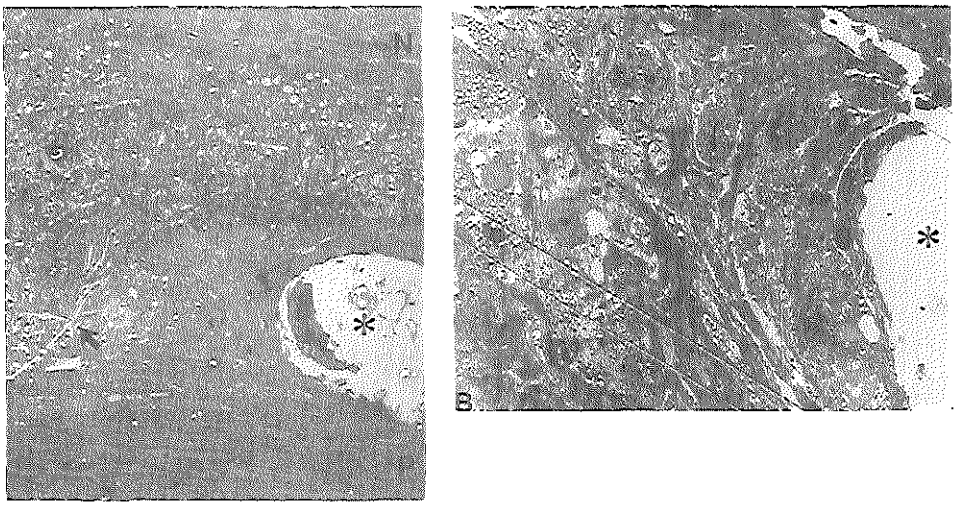
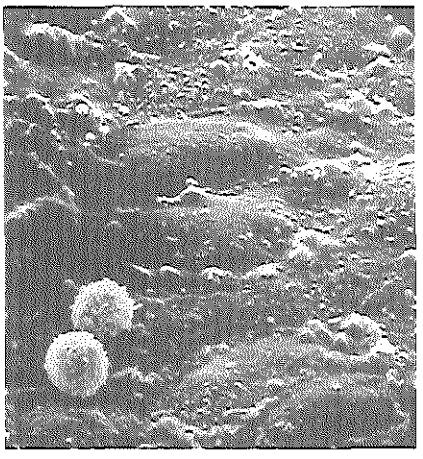


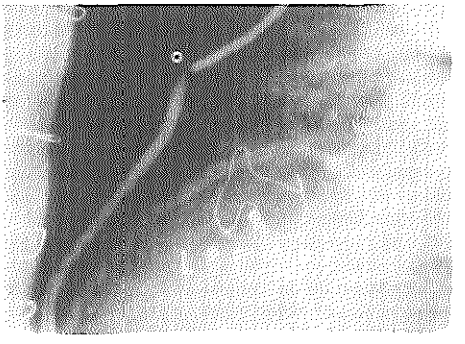
Figure 6. A, Transmission electron micrograph of saphenous vein bypass graft 6 months after stent implantation. Extracellular lipid deposits and cholesterol clefts (arrow) are evident alongside the foam cells. B, Higher magnification of another part of the section in A. An abundant number of foam cells can be seen. The diagonal lines are artifacts produced by the ultramicrotome. (*) = void representing the site of a removed 70 μm diameter stent wire. Abbreviations as in Figure 5.

Figure 7. Scanning electron micrograph of the endothelial lining shows an irregular, raised endothelial surface. Two leukocytes are adherent to the endothelium (bar = 5 μm).



foreign body in the vascular wall will continue to stimulate fibrointimal hyperplasia after the 6 months usually associated with balloon injury is also unknown. Concern has also been expressed as to whether the stent can trigger an allergic response, particularly in individuals who are hypersensitive to the individual metals that make up the device. Although there have been reports of transient inflammatory infiltrates in the adventitia after stent implantation, it is reassuring that there have been no reports of foreign body cells in the immediate vicinity of the implanted device in the experimen-

Figure 8. Coronary angiogram from a 67 year old patient who underwent stent implantation for a severe narrowing in the shaft of a 10 year old saphenous vein bypass graft. Three months later he was treated with atherectomy for restenosis within the stent.



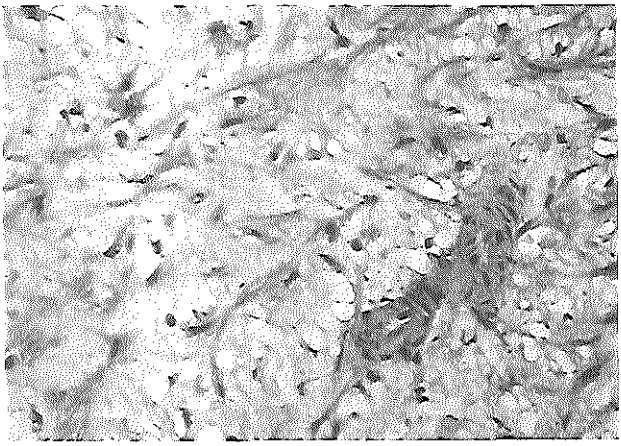


Figure 9. Light micrograph of hyperplastic tissue removed by atherectomy from the narrowed stent in Fig. 8. The specimen contains abundant smooth muscle cells and loose extracellular matrix. (Hematoxylin-eosin stain.)

tal animal model (14,15). This has been confirmed in limited experience in retrieved human stented coronary bypass grafts. In vitro attempts (Van der Giessen et al., unpublished observations) to identify endothelial membrane lipid peroxidation by free radicals formed in the presence of metallic stent elements have been unsuccessful, although the theoretic possibility exists.

2. Long-term effects of continuous barotrauma: role of exerted radial pressure. The chronic effects of continuous barotrauma induced by the expanded stent may have important ramifications. Because the Wallstent's properties are analogous to those of any spring, it tries to assume its equilibrium configuration, defined as the unconstrained diameter where net radial force is zero. If it is stretched beyond or constricted below this equilibrium, it generates forces to return to this configuration. We have studied the in vitro force-length relationship of the Wallstent stent (Fig. 11). These measurements have yielded calculations of the radial pressures exerted by this stent, both globally and locally at the site of the individual filaments if the stent is

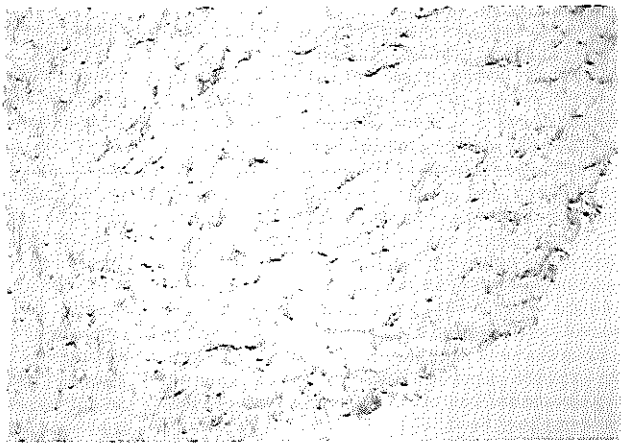


Figure 10. Smooth muscle cells are identified in the restenosis tissue obtained at atherectomy by the dark brown staining. An antibody specific for smooth muscle alpha actin has been coupled to a peroxidase reaction and is responsible for the dark color.

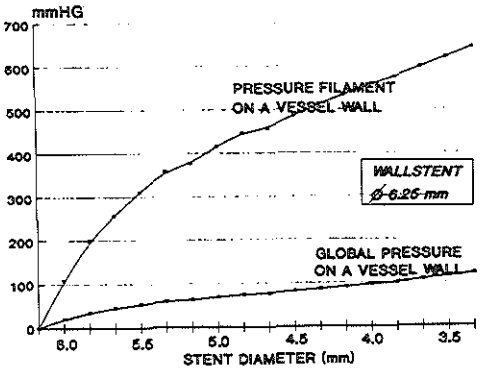


Figure 11. Radial pressures of the individual stent filament and the global pressure on the vessel wall of a self-expanding Wallstent (unconstrained diameter 6.25 mm) at varying degrees of expansion.

maintained at a diameter less than that of the unconstrained diameter. Significant pressures are generated by the stent (as in any spring-loaded device) to return it to its unconstrained size. For example, an unconstrained 6.25 mm diameter stent generates a radial global pressure of 50 mm Hg and a radial local pressure of about 300 mm Hg at the stent filament if it is maintained at 5.5 mm. This increases to 90 mm globally and 500 mm locally at 4.5 mm diameter. These pressure calculations would be additive to the mean arterial pressure and could have an important impact in situations where oversized Wallstents are implanted. In fact, localized areas of necrosis adjacent to the stent wires have been seen, which are probably the result of a pressure phenomenon.

3. Splinting the artery externally (casting) versus internally (stenting): effect on wall stress. Parallels have been drawn between the effects of splinting the artery externally (casting) and internally (stenting). Thubikar et al. (66) showed that externally casting segments of rabbit aortas limits pulsatile flow and atheroma development despite a high cholesterol diet. On the basis of this observation, others (32) have speculated that nonflexing internal stents may also reduce wall stress and consequently diminish hyperplasia formation. However, this analogy is not impressive. Although some of the pulsatile stretch may be borne by the external cast or the internal stent, and thus favorably affect wall stress, this is achieved by separate means. In the casting model, there should be a reduction in intramural wall stress, since the vessel is casted at a radius smaller than the maximal systolic expansion. In contrast to casting, stenting results in dilation of the artery and an increase in wall stress. This important stimulus to intimal hyperplasia appears to overcome the inhibitory effects of reduction in phasic vessel wall expansion. Booth et al. (67) modified the external casting model with interesting results. By applying an external nonoccluding Silastic collar on a rabbit carotid artery that did not affect end-systolic dimensions, they demonstrated that focal hyperplastic lesions rapidly develop. This finding supports the concept that external casting must decrease the vessel radius in order to achieve inhibition of intimal hyperplasia.

The State of Interventional Cardiology in 1990: Debulking Versus Dilating

Interventional cardiology has moved in two directions: devices that primarily dilate coronary narrowings (balloon angioplasty and stenting) and devices that physically debulk coronary tissue by extraction, liquefaction or vaporization (laser, directional and rotational atherectomy and spark erosion). At present, it is difficult to make comparisons among the various devices. However, there are several fundamental differences that may be important and merit further discussion.

Comparison of the various techniques. The ideal coronary intervention should selectively reduce the effect of the atheromatous lesion with minimal alteration of the normal vessel wall components and architecture. None of the currently available techniques completely satisfy these requirements. Balloon angioplasty, atherectomy (rotational and directional) and laser devices all cause extensive traumatic changes within the plaque and usually major alterations to the vessel wall architecture as well. Balloon angioplasty, the earliest intervention, has been shown to create tears and dissections within and at the edges of atherosclerotic plaques and frequently disrupts the internal elastic membrane and medial layers (68–71). Theoretically, these disruptions may be advantageous since the liberation of lipid and debris from the atheromatous lesions, a sort of debridement, may favorably affect the long-term biologic growth and behavior (if distal embolization of this material does not cause immediate clinical consequences) (72–74).

However, more important considerations may be the manner in which the healing process ensues in a damaged vessel with frayed, ragged membrane edges and separated muscular layers and the inherent problems of restoring the normal three-layered architecture of the arterial wall in an orderly fashion after such injury. Moreover, the extent of arterial disruption from angioplasty appears to be much less than in the actual removal of coronary tissue by debulking devices. Directional atherectomy in particular has been shown to be extremely effective in removing the atheroma but specimens include adventitia in up to 30% of cases (75), although there is no evidence to date that the rate of restenosis is related to the depth of the vessel wall extracted. Alternatively, stenting seems to be the least disruptive to the underlying architecture, although the underlying atheromatous lesion persists in the stented vessel with unknown future consequences. Stenting is able to "tack back" the cracks and tears induced by balloon angioplasty, which may

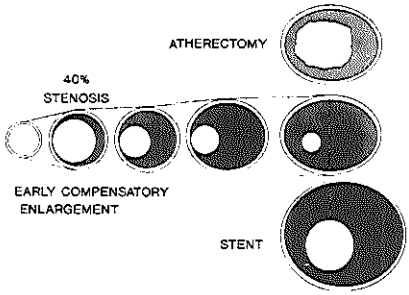


Figure 12. The natural progression of coronary artery disease as suggested by Glagov et al. (76) is illustrated in the **center row** by the early compensatory enlargement and the late luminal narrowing of progressive atherosclerosis. The differential effects of stenting and atherectomy in restoring the vessel diameter are shown. Stenting **(below)** restores the early compensatory enlargement in the vessel while it maintains the basic architecture of the vessel wall. By extracting vascular tissue, atherectomy **(above)** disrupts the underlying architecture of the wall. Modified from Glagov S et al. (76) with permission.

diminish the stimulus for fibrosis in much the same way that a properly closed wound minimizes fibrotic scar in the healing phase.

Glagov revisited. Glagov et al. (76) observed that the diseased coronary artery is able to adapt to progressive plaque expansion by enlarging the size of the vessel. This compensatory mechanism maintains the luminal area until the plaque lesion occupies 40% of the area inside the internal elastic lamina, beyond which progressive luminal narrowing occurs. In other words, significant atherosclerosis can coexist with normal or even enlarged luminal area until the limits of this adaptation are exceeded.

Striking similarities exist between the chronic process of atherosclerosis and the situation in the stented vessel wall. The stent is initially embedded in the intima, which results immediately in enlargement of the lumen and later in localized medial thinning at the site of the stent wires, a commonly observed pathologic feature of atherosclerosis. Stenting may be regarded as the invasive cardiologist's attempt to restore the aforementioned "Glagovian" balance between plaque and luminal area, but in vessels that contain plaques >40% of the internal elastic lamina (Fig. 12). Stents effectively alter the relation between plaque size and lumen area, resulting in a shift in the curve. Progressive vessel dilation by the stent can maintain adequate luminal area unless excessive fibrointimal hyperplasia upsets the new balance.

Expansion ratio. Expansion ratio is an important concept that relates the final effect on the arterial diameter to the size of the catheter required to deliver this effect (32) (Table 1). A

Table 1. Expansion Ratios With the Coronary Interventional Devices

Intervention	Procedures	Device Profile (mm)	Vessel Diameter Preprocedure (mm)	Maximal Achievable Diameter/(Range) (mm)	Postprocedure Diameter (mm)	Theoretic Expansion Ratio	Effective Expansion Ratio
Balloon angioplasty	443	0.7–1.3	1.1 ± 0.3	2.9 ± 0.4 (2.0–3.5)	1.8 ± 0.4	2.2–4.1	1.4–2.6
Stenting							
Self-expandable	357	1.6	1.3 ± 0.7	4.0 ± 0.7 (2.5–6.0)	2.6 ± 0.6	2.5 (1.6–3.8)	1.6
Balloon-expandable	27	1.4–1.6	1.0 ± 0.3	3.3 ± 0.3 (3.0–4.0)	2.4 ± 0.3	2.1–2.4	1.5–1.7
Atherectomy							
Directional	39	2.1–2.5	1.1 ± 0.4	3.3 ± 0.5* 2.0 ± 0.2†	2.5 ± 0.6	1.3–1.6	1.0–1.2
Rotational	52	1.5–2.0	0.9 ± 0.3	1.9 ± 0.3 (1.5–2.0)	1.7 ± 0.4	1.0	0.9–1.1
Excimer laser	55	1.4	0.5 ± 0.4	1.4	1.7 ± 0.5	1.0	1.2

*With balloon inflated; †with balloon deflated. This table compares the device profile and immediate angiographic results of several interventions. The profile of the device is based on data on 2.0 to 3.5 mm diameter balloon catheters (77), the Wallstent (Medinvent) self-expandable stent, Wiktor (Medtronic) balloon-expandable stent, Simpson Coronary Atherocath (DVI) directional atherectomy device, Rotablator (Heart Technology) rotational atherectomy device and the model Max-10 excimer laser (Technolas, Munich). The relation between the profile of the device and the maximal achievable diameter of the device is the theoretical expansion ratio. The maximal achievable diameter of the vessel is calculated according to the size of the device while it is operational in the coronary vessel. In the case of balloon angioplasty, balloon-expandable stent and directional atherectomy, the maximal achievable diameter corresponds to the diameter of the device while the balloon is inflated and to the unconstrained diameter of the self-expandable stent. The rotational atherectomy device and the excimer laser do not alter their diameter during the procedure. The postprocedure diameter is measured immediately after the procedure. The effective expansion ratio represents the ratio between the postprocedure result and the profile of the device and thus indicates not only the initial effect of the device but also the effect of elastic recoil, which is primarily responsible for the deterioration in the diameter from the maximal achievable diameter to the postprocedure diameter. The diameter values listed are the mean value ± SD of the different-sized devices from each interventional study; ranges are in parentheses. The preprocedure data, which may also affect the postprocedure result, were similar for all interventions (0.9 to 1.3 mm) except for the excimer laser, which may explain a somewhat lower postrecoil diameter and effective expansion ratio. The quantitative angiographic data for all devices except the rotational atherectomy device (Peterson K, unpublished observations) and the excimer laser (79), were collected at the Thoraxcenter.

favorable ratio is best exemplified by a small catheter delivery system that is able to pass severely narrowed segments and yet optimally dilate the stenosis. The maximal effect of the device may be partially lost because of the elastic recoil of the vessel. The current interventional devices may have differential effects in these two areas: the immediate result when the device is initially used, and then the partial loss of the initial gain after the device has been removed. An attempt has been made to separate these two effects by subdividing the expansion ratio into the theoretical expansion ratio (a measure of the effect while the device is operational) and the functional expansion ratio (which takes into account the elastic recoil phenomenon). For example, a 4 mm diameter balloon angioplasty catheter should achieve a vessel diameter of 4 mm at the time of balloon inflation but this is reduced immediately after deflation, primarily because of the elastic recoil of the vessel. Balloon angioplasty and stenting give extremely favorable theoretic and effective expansion ratios since they may be delivered on low profile catheters. The wide range for the theoretic and effective expansion ratios seen with balloon angioplasty is explained by the variation in the size of the balloons (2.0 to 3.5 mm) used in the study from which these data were obtained. The atherectomy devices are more limited by the profile of the device that is introduced into the coronary artery. The dimensions of the rotational atherectomy device and the excimer laser do not change while in operation and therefore both exhibit lower theoretic expansion ratios. However, by physically removing or vaporizing tissue, the potential elastic recoil effect is diminished by atherectomy and excimer laser devices.

Conclusion

Once again the question is asked: Can the promises of the new technology, and in particular the coronary stent, ever be realized?. Stenting will only achieve clinical acceptance when the safety, efficacy and cost efficiency are superior to those of balloon angioplasty alone. Safety remains the major limitation of stenting. In the initial 105 patients with an implanted Wallstent, 20% had documented occlusion within the 1st 14 days, usually resulting in myocardial infarction and in some cases necessitating emergency bypass surgery. With further experience this was reduced to 13% in the next 100 patients. Schatz et al. (15) recently reported a 3.6% subacute occlusion rate in contrast to a 16% rate in their early experience when warfarin treatment was omitted (78). However, the price of chronic anticoagulation therapy—bleeding complications, prolonged hospitalization to initiate therapy and effects on the quality of life—must also be considered. With the increasing importance of third party payment, the cost differential among competing therapies will also dictate medical policies. Finally, these devices must show beneficial effect on late restenosis in order to gain clinical acceptance. The late follow-up results of quantitative coronary angiography for two of the stents will be published in the next several months and should provide more objective evidence with which to evaluate the future role of the stent.

The early experience of modern interventional cardiology evokes lessons contained within Pandora's box. In this classic Greek myth, man, beguiled by the attraction of Pandora opened the box that exposed heretofore unknown perils and disease, while Hope (Elpis), the lone content of the box that could be controlled by man, remained hidden deep within the box. The allure of the newer techniques and devices in interventional cardiology, like Pandora herself, have brought us a new set of problems that we have been ineffectual in solving. However, Hope also exists within the modern Pandora's box and our capacity to realize hope will depend on a scientific approach to the problems of restenosis and neointimal hyperplasia in a mutual effort (in concert with industry) of interventional cardiologists, pathologists, molecular biologists, biochemists, pharmacologists and our patients, the general public.

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Chapter 3

Do stents interfere with the densitometric assessment of a coronary lesion? An in-vitro study

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Cardiovasc Cathet Diag 1991 (in press)

Abstract

This in-vitro study was designed to assess the contribution of 3 currently investigated coronary stents to the densitometric measurement of a known stenosis contained within two different sized plexiglass phantoms. These studies were performed at two concentrations of the contrast agent iopamidol (50 and 100%). The calculated minimal luminal cross-sectional area values in the control phantom ranged from 0-18% higher than the theoretical values. Insertion of a stainless steel stent (Wallstent^{R} or Palmaz-Schatz^{TM}) resulted in further minor increases (≤8%) in the calculated minimal luminal cross-sectional area, except in the smaller phantom filled with 50% contrast medium. The Wiktor^{TM} (tantalum) stent had the largest impact of the three stents depending on the concentration of iopamidol (100% contrast medium: 9-13% values above control; and 50% contrast medium:, 23-56% higher). We conclude that although densitometry may overestimate the minimal luminal cross-sectional area in stented vessels, this effect is usually minor with stainless steel stents. However tantalum-containing stents may result in serious overestimation of lesion area, particularly if contrast is diluted or the vessel is not well filled.

INTRODUCTION

Coronary stenting has been introduced as an adjunct or alternative to PTCA in obstructive coronary artery disease (1,2). A variety of devices have been developed which differ in their composition, design, radiopacity and mode of expansion. Although densitometry and contour detection appear to be comparable methods to assess the angiographic result after stenting with the Wallstent (3), the actual contribution of the individual types of stent to the densitometric value has not been systematically studied. The objective of this study was to assess whether the physical properties of three devices currently under investigation affect the densitometric assessment of a fixed stenosis in a plexiglass phantom.

MATERIALS AND METHODS

Plexiglass Phantoms

Plexiglass models were 6 cm in length and circular in cross-section. The extremities of the phantoms had a fixed bore diameter of either 4 or 3 mm and abruptly tapered in the central part to a fixed concentric stenosis of 3 and 2 mm respectively.

Stent Types

The Wallstent^{R} (Schneider, Zürich) is a self-expandable stainless steel woven mesh prosthesis, constructed of 16 wire filaments, each 0.07 mm. wide. It is constrained in an elongated configuration on delivery catheter with the distal end covered by a removable plastic sleeve. The combined diameter of delivery catheter plus mounted stent measures 1.57 mm diameter. As the sleeve is withdrawn, the constrained device returns to its original unconstrained larger diameter and becomes anchored against the vessel wall (1,2). Unconstrained stent diameter in this study was 2.5 mm in the 2 mm phantom and 3.0 mm in the 3 mm phantom.

The Palmaz-Schatz^{TM} coronary stent (Johnson and Johnson, Warren, New Jersey) is a balloon expandable slotted stainless steel tubular stent composed of two 7 mm segments of slotted tubes that are connected by a 1 mm bridging strut (4). Each tube contains 12 rows of slots that are each 0.065 mm in diameter. The stent is mounted coaxially over commercially available balloon catheters. In this study the stent was cut at the bridging strut and only the tube was placed in the phantom.

The Wiktor^{TM} stent (Medtronic, Minneapolis) is a balloon expandable tantalum helical coil stent that is constructed of a single tantalum wire (0.127 mm in diameter) which is formed into a sinusoidal wave and wrapped into a helical coil structure. The prosthesis is crimped onto a deflated polyethylene angioplasty balloon and the maximal diameter of the balloon after inflation determines the ultimate size

of the prosthesis after implantation (5).

Angiographic Protocol

Angiograms were obtained with the use of a Siemens Bicor X-ray system in the 5 inch cesium iodine image intensifier mode and a 0.8 mm focal spot of the X-ray tube. The focus to object distance was 90 cm and the object to image intensifier distance was 13 cm. In each study, the stent was introduced into the plexiglass phantom and positioned across the narrowing at the center of the phantom. All three stents completely covered the central narrowing and extended on either side to the reference segment. The phantoms were then filled with contrast medium, Iopamidol-370, at a concentration of either 50% or 100%.

Cinefilm was taken with an additional 17.5 cm thickness of plexiglass blocks (12.5 cm anterior and 5 cm posterior to the models). The addition of the plexiglass blocks resulted in a more appropriate kV level (82 kV) and in a scatter medium which more closely approximates the radiologic scatter in the human thorax during angiography. The studies were then repeated with the second concentration of the contrast reagent. The cinefilms were processed routinely and analyzed by the CAAS system.

Angiographic Analysis

All cineangiograms were analyzed using the computer assisted cardiovascular angiography analysis system (CAAS) which has previously been discussed in detail (6-9). The important steps will be briefly described. Any area of size of 6.9 X 6.9 mm in a selected cineframe (overall dimensions 18 X 24 mm) encompassing the phantom or the desired arterial segment can be digitized by a high resolution CCD-camera with a resolution of 512 X 512 pixels and 8 bits of gray level. Contours of the "vessel" segments were determined automatically based on the weighted sum of the first and second derivative functions applied to the digitized brightness information along scanlines perpendicular to the local centerline directions of the perspex model. The centerline was manually defined by the user. A computer-derived estimation of the original dimension at the site of the narrowing is used to define the interpolated reference diameter. This technique is based on a computer-derived estimation of the original diameter values over the analysed region (assuming there was no narrowing present) according to the diameter function. Calibration of the diameter data is performed by applying the automated contour detection technique to the known outer diameter of the plexiglass model.

The densitometric assessment, which relies on the relationship between pathlength of the X-rays through the segment and the resulting brightness values, requires a detailed analysis of the complete X-ray/cine/video chain, including the film development process.

For the first part of the chain, from the X-ray source to the output of the image intensifier, we use Lambert Beer's law for the x-ray absorption and apply certain models for the x-ray source and the image

intensifier. From the output of the image intensifier up to the brightness values in the digital image, a simple linear transfer function was used. Details of this technique have been described elsewhere (6-8). The cross-sectional area of the perspex narrowing is then obtained as follows. Contours of the segment are detected by automated contour detection with the CAAS system as previously described. From the measured diameters along the analysed segment, the previously described diameter function is derived. On each scanline perpendicular to the local centerline direction of the vessel, a profile of brightness values is measured. This profile is transformed into an absorption profile by means of a simple logarithmic transfer function to correct for the Lambert-Beer Law. The background contribution is estimated by computing the linear regression line through the background points directly left and right of the detected contours. Subtraction of this background portion from the absorption profile within the perspex contours yields the net cross-sectional absorption profile. Integration of this function gives a measure for the cross-sectional area at the particular scanline. By repeating this procedure for all scanlines, the cross-sectional area function is obtained. An absolute reference densitometric area value can then be obtained by assuming a circular configuration in the reference segment and using the diameter measurements obtained from the diameter function. Figure 1 shows an example with the computed diameter function curve (upper curve) and area function curve (lower curve) displayed upon the video image. The minimal luminal cross-sectional area can then be calculated by the ratio of the absolute difference in brightness of the reference area and the narrowed segment, with the known cross-sectional area of the reference segment. The complete procedure has been evaluated with the cinefilms of perspex models of coronary obstructions that did not contain stents. In this protocol, one frame from each study was analysed at least three times.

RESULTS

The densitometric assessment of the plexiglass models containing the stents is shown in Table 1. The theoretical minimal luminal cross-sectional areas of a 2 mm and 3 mm diameter phantom are 3.14 and 7.06 mm^2, respectively. In the control phantom, the calculated minimal luminal cross-sectional area values ranged from 0-18% higher than the theoretical value in the 3x2 phantom, and was 8% higher in the 4x3 phantom (Figure 1a). Only minor increases in minimal luminal cross-sectional area (≤8%) were noted in the phantoms that contained the Wallstent and Palmaz-Schatz stents compared with the control phantom except for the 3x2 phantom filled with 50% contrast medium (13 and 25% higher respectively). The minimal luminal cross-sectional area in the phantom containing the Wiktor stent was moderately higher (9-13%) than the control with 100% contrast medium. However, in the presence of 50% contrast medium, the Wiktor stent, had the most adverse effect (23-56% higher than control values) in the determination of the minimal luminal cross-sectional area. This effect was most pronounced in the smaller 3x2 phantom (Figure 1b).

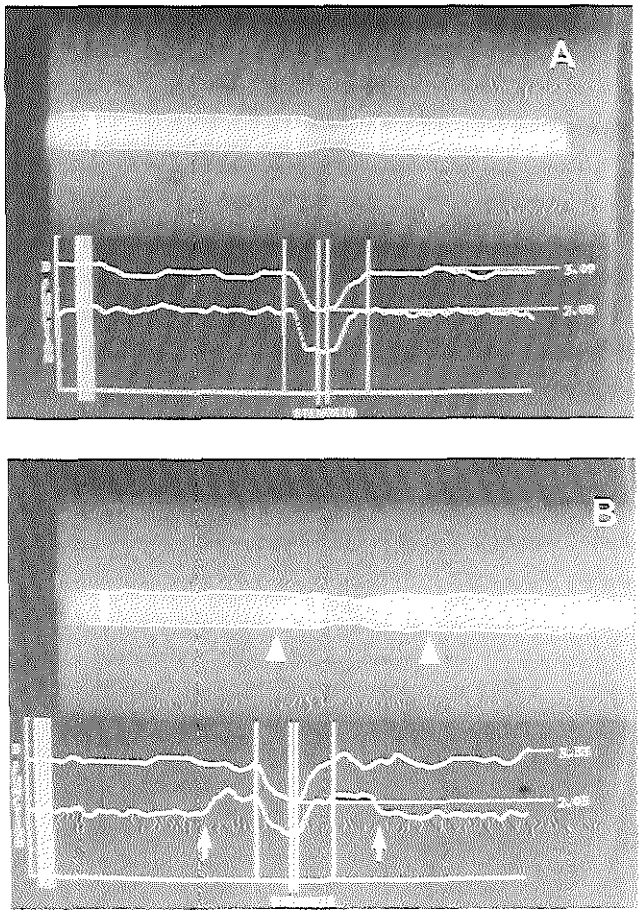

Figure 1. *Control (A) and Wiktor-containing (B) plexiglass phantoms (3x2 mm) filled with 100% and 50% iopamidol contrast reagent respectively. Graphs show the diameter function (upper curve) and the densitometric area function (lower curve). Outside vertical lines on the graph and rightward two vertical lines on the phantom are lesion boundaries. The inner two vertical lines represent the minimal points on the diameter and densitometric graphs respectively. The multiple vertical lines in the left part of the graph and the leftward vertical line in the phantom represent the user defined reference segment. The numbers in the graph represent the maximum and minimum diameter. The boundaries of the Wiktor stent are visible in the phantom (arrowheads) and as a step-up in the densitometry graph (arrows). As a result of the Wiktor stent contribution to the densitometry values, the minimal cross-sectional area determination is overestimated compared with a control phantom.*

DISCUSSION

This study illustrates two important points. First, in these particular models, densitometric determinations of minimal luminal cross-sectional area in control phantoms using the CAAS system are

overestimated. In most of the study conditions, this is a minor effect except for the smaller diameter phantom at 100% concentration. This degree of error is in concordance with Whiting et al (10). Secondly, the addition of stents further contributes to an even spuriously higher measurement of the minimal luminal cross-sectional area, particularly with the Wiktor stent.

The radiographic assessment of coronary stents includes several important features. During the procedure, the ability to visualize the stent is important for proper stent deployment to ensure adequate coverage of a lesion. The Wiktor™ stent has a decisive advantage since tantalum is much more radiopaque than stainless steel, particularly at kv levels used in angiographic studies of the human thorax (Figure 2). Densitometry has a theoretical advantage over edge detection since it is a more direct measurement of stenosis cross-sectional area. This parameter appears to be more closely related than vessel diameter to coronary flow of hemodynamics (11). Thus densitometry may be an important method to evaluate the immediate angiographic result post stenting, and is particularly useful to analyse eccentric lesions since it is theoretically independent of the geometric shape. Although contour detection appears to be equally acceptable in Wallstent implanted lesions (3), it has been unreliable in some cases of Wiktor implantations due to detection of the radioopaque stent wires as the border instead of the narrowing within the stent (Figure 3).

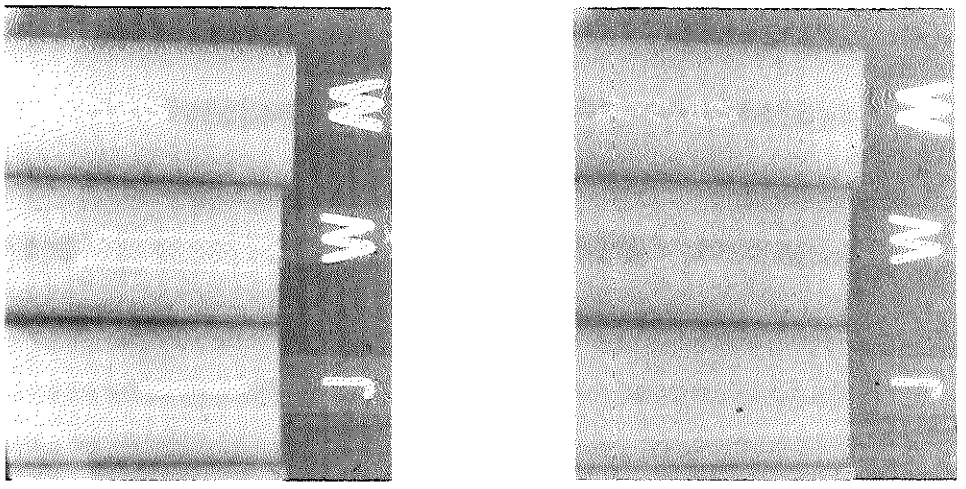

Figure 2. *Radiopacity of three stents at two kilovoltage levels in plexiglass phantoms without contrast. (left) 50 kv, (right) 82 kv. J= Johnson and Johnson (Palmaz-Schatz™), W= Wallstent®, M= Medtronic (Wiktor™). The Palmaz-Schatz™ stent was not expanded in the phantom.*

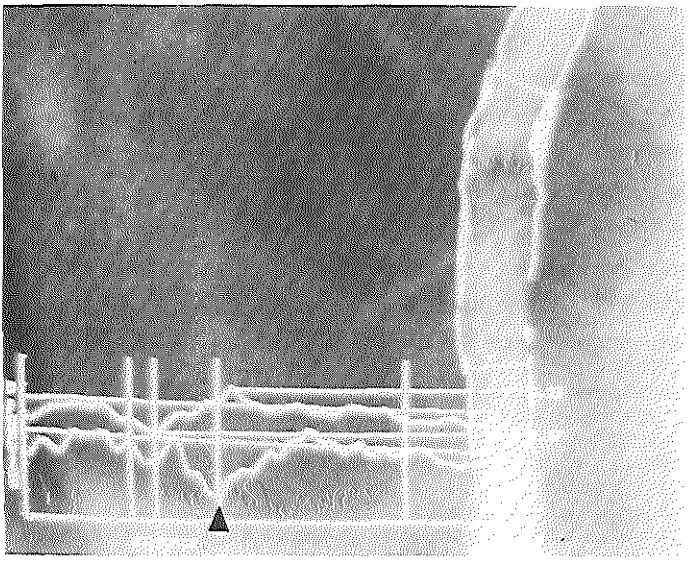

Figure 3. *Edge detection and densitometric analysis of an obstruction within a Wiktor™ stent in the right coronary artery. The four vertical lines in the graph were explained in Figure 1. The boundaries of the stent are represented by the two horizontal lines in the angiographic image and the two outer vertical lines in the graph. A discrepancy between the two graphs is present and most severe at the vertical line which denotes the minimal densitometric value (arrowhead). The edge detection follows the outline of the stent and does not recognize the stenosis within the stent.*

This study suggests that densitometric assessment of Wiktor-containing lesions is also affected by the radiographic properties of this particular stent, especially in situations where the contrast medium is diluted or the vessel is not well filled. This effect consistently resulted in an overestimation of the minimal luminal cross-sectional area by 9-56% from control values, depending upon the study conditions. Although the smallest differences between the Wiktor containing phantoms and control phantoms occurred with 100% contrast medium, these are also the conditions that resulted in the largest discrepancy between the control phantoms and the true values. Thus, the overall error in Wiktor containing phantoms with 100% contrast medium compared with the true value was in the range of 18-33%. At a lower contrast reagent concentration, the overestimation of minimal luminal cross-sectional area in the Wiktor stent was more pronounced compared with the control phantom, especially in the 2 mm phantom narrowing since there is relatively less contrast (and thus less iodine) present in the smaller phantom in comparison to the amount of tantalum contained in the stent wires. Therefore, the contribution of the stent wires to the overall densitometric value is more significant and results in the higher calculated values of the minimal luminal cross-sectional area. This may have important consequences in follow-up cases of restenosis where the vessel lumen may be critically reduced in calibre.

The radiopacity of a stent is affected by several variables including the composition of the material (and its atomic number), the total surface area of the wires in the stent and the cross sectional area of the stent wires encountered by the X-ray beam in any particular projection. The Wiktor stent is made of radiopaque tantalum which, due to its higher atomic number than stainless steel has a higher attenuation constant and thus increased radiopacity than the two stainless steel containing stents. X-ray energy dispersion spectrometry studies were done in all 3 stents to determine if other metals were present that could alter the attenuation coefficient (Figure 4). The Wiktor™ stent contained only tantalum. The composition of the Wallstent and the Palmaz-Schatz stent were very similar except for the presence of cobalt in the Wallstent®. However, this would only minimally affect the attenuation constant.

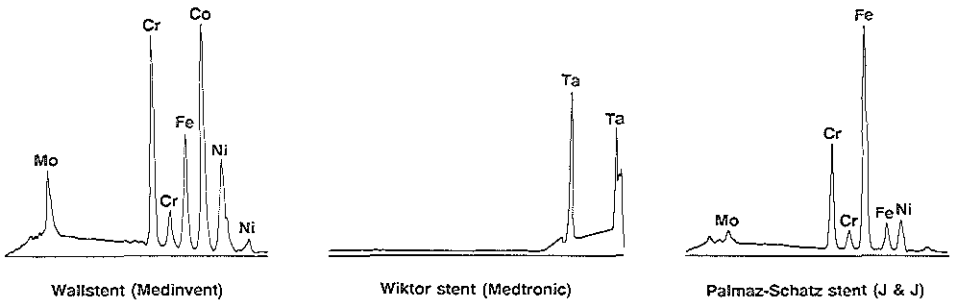

Figure 4. *X-ray energy dispersion spectometry identifies the composition of the metals used in each of the three stents. Mo = Molybdenum, Cr = Cromium, Co = Cobalt, Fe = Iron, Ni = Nickel, Ta = Tantalum*

The cross sectional wire area of the stent is dependent on the number and thickness of the wire(s) at any particular point along the stent. This is more difficult to estimate in the Wiktor™ stent due to the asymmetric and helical design of the stent and the orientation of the wire loops. Consequently, the cross-sectional area of the stent is not uniform at each segment over the length of the stent. It is estimated that at any one segment, a cross-section could contain from 4 to 8 cut wires with a probable average of 6 wires. This corresponds to a cross-sectional area of the stent ranging form .0005 cm^2 to .001 cm^2, and an average value of .00075 cm^2. The total wire cross-sectional area of the Wallstent and Palmaz-Schatz stent, which are easier to calculate due to the more uniform design of each stent, are 0.00062 cm^2 and 0.00039 cm^2 respectively.

Since the composition and total wire cross-sectional area of the two stainless steel stents are nearly identical, it is not surprising that these two stents had a similar effect. This effect in general was quite consistent and minimal. Although the largest discrepancy between stainless steel stented and control phantoms occurred in the smaller phantom at 50% contrast concentration, these were the exact conditions that no overestimation of minimal luminal cross-sectional area occurred in the control

phantoms compared with the true value.

In conclusion, densitometry may overestimate the minimal luminal cross-sectional area in stented vessels. Relatively minor and probably clinically insignificant changes occur in the Palmaz-Schatz stent and the Wallstent. However densitometric analysis of lesions containing the Wiktor stent, particularly if the vessel is not well opacified, will result in serious overestimations of the minimal luminal cross-sectional area. Clinical trials that compare various stents or stenting to PTCA using densitometry should consider this effect in tantalum containing stents.

Table 1. Videodensitometric determination of minimal luminal cross-sectional area in plexiglass phantoms containing no (control) or 3 different metallic stents. (%) = % difference between the stented and control phantoms.

Iopamidol Concentration	Reference Diameter	Stenosis Diameter	Minimal Luminal Cross-Sectional Area (mm^2)				
	(mm)	(mm)	True	Control	Palmaz-Schatz	Wallstent	Wiktor
100%	3	2	3.14	3.70±.10	3.55±.10 (-4%)	3.93±.11 (+6%)	4.17±.23 (+13%)
	4	3	7.06	7.61±.30	8.19±.25 (+8%)	7.78±.50 (+2%)	8.33±.21 (+9%)
50%	3	2	3.14	3.14±.13	3.91±.31 (+25%)	3.54±.07 (+13%)	4.89±.08 (+56%)
	4	3	7.06	7.65±.21	7.92±.07 (+4%)	7.81±.50 (+2%)	9.40±.38 (+23%)

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Chapter 4

Edge detection versus densitometry for assessing coronary stenting quantitatively

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Edge Detection Versus Densitometry for Assessing Coronary Stenting Quantitatively

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The optimal method used to analyze quantitatively the immediate angiographic results of coronary stenting in the coronary arteries has not been studied. Accordingly, minimal luminal cross-sectional area was determined by 2 methods, edge detection and densitometry, in 19 patients who underwent percutaneous transluminal coronary angioplasty (PTCA) and then coronary stent implantation for symptomatic coronary stenoses. The correlation coefficient, 0.73 before angioplasty, decreased to 0.59 after coronary angioplasty and then increased to 0.83 after stent implantation. The mean differences between edge detection and densitometric determinations of minimal luminal cross-sectional area were 0.31 ± 0.51 mm² before PTCA, −0.38 ± 1.22 mm² after angioplasty and 0.35 ± 0.79 mm² after coronary stenting. It is concluded that, although the correlation and variability in the measurement of minimal luminal cross-sectional area between edge detection and densitometry deteriorate after PTCA, they are improved after stenting, probably because of smoothing of the vessel contours by the stent and remodeling of the stented segment into a more circular configuration. Therefore, in the stented coronary artery, edge detection and densitometry are equally acceptable methods of analysis.

(Am J Cardiol 1991;67:484–490)

Stenting of the coronary arteries is currently being investigated as an adjunct to percutaneous transluminal coronary angioplasty (PTCA).[1] The optimal method used to analyze the immediate angiographic results of stenting in the coronary arteries has not yet been determined and is part of a general and unsettled controversy in the immediate assessment of PTCA. Computer-based automatic edge detection angiographic analysis systems have reduced the variability resulting from visual and caliper-determined contour detection,[2–4] but their use may be limited in eccentric lesions, particularly after angioplasty, when acute tears and dissections additionally distort the anatomy. Densitometry has been proposed as an alternative method of angiographic assessment of the severity of coronary obstructions because it is independent of the geometric shape.[5,6]

The hemodynamic significance of a lesion has previously been shown to be most closely correlated with the minimal cross-sectional area.[7,8] The determination of this parameter from edge detection programs from a single projection requires an assumption, often incorrect, that the vessel cross section is circular.[9,10] Our group has previously shown that discrepancies exist in the postangioplasty analysis between edge detection and videodensitometric methods, although conflicting data have also been published.[5,11,12] However, the situation after stenting of the coronary arteries may be altered, because the arterial wall typically assumes a smoother, more circular appearance. We therefore undertook this study to determine if stenting of coronary arteries after PTCA improves the correlation and agreement between videodensitometry and edge detection methods.

METHODS

Study patients: Nineteen patients, 13 men and 6 women, ranging in age from 41 to 70 years (mean 56), were enrolled after giving informed consent for stent implantation. The dilated and stented coronary artery was the left anterior descending coronary artery in 12 patients, the circumflex coronary artery in 2, the right coronary artery in 3 and a coronary artery bypass vein graft in 2. This series consisted of the first 19 patients in whom edge detection and videodensitometry were used to evaluate the immediate results of the procedure. In each patient, the coronary artery stenosis was dilated first. After successful angioplasty, the balloon catheter was exchanged for the stent delivery system over a 0.014-inch exchange guidewire. Unconstrained stents of 15 or 20 mm in length, depending on the lesion, were

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TABLE I Quantitative Angiographic Data

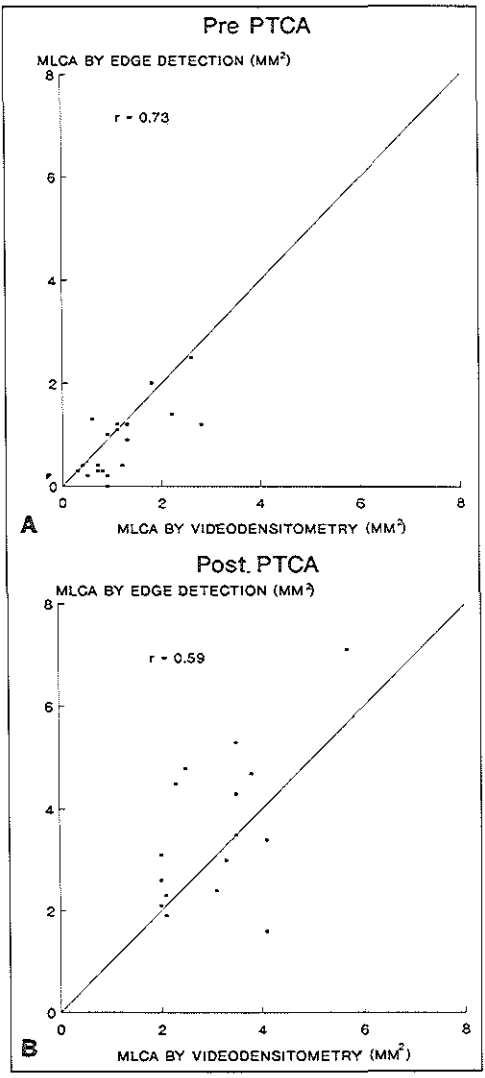
Pt. No.	Age (yr) & Sex	PTCA Vessel	Obstruction Diameter (mm)	Diameter Stenosis (%)	Minimal Luminal Cross-Sectional Area: Edge Detection (mm^2)	Minimal Luminal Cross-Sectional Area: Densitometry (mm^2)
1 PreP	70M	Conduit	0.9	67	0.7	0.4
PostP			2.2	24	3.8	4.7
PostS			3.0	8	7.1	7.1
2 PreP	70M	Conduit	1.2	55	1.2	0.4
PostP			1.6	22	2.1	2.3
PostS			2.3	10	4.1	2.3
3 PreP	52M	Right	1.8	50	2.6	2.5
PostP			2.1	46	3.5	5.3
PostS			2.8	23	6.1	5.7
4 PreP	42M	LAD	1.0	54	0.9	0.2
PostP			1.6	31	2.1	1.9
PostS			1.8	23	2.5	2.7
5 PreP	52M	LAD	1.3	47	1.3	0.9
PostP			—	—	—	—
PostS			1.7	26	2.3	1.1
6 PreP	46M	Right	1.0	67	0.7	0.3
PostP			1.8	46	2.5	4.8
PostS			2.5	28	4.9	5.7
7 PreP	69M	LAD	1.1	62	0.9	1.0
PostP			1.6	40	2.0	2.1
PostS			2.1	28	3.5	3.0
8 PreP	64F	LAD	1.5	48	1.8	2.0
PostP			2.0	26	3.3	3.0
PostS			2.2	21	3.8	3.6
9 PreP	62F	LAD	0.7	73	0.4	0.4
PostP			—	—	—	—
PostS			2.2	16	3.8	4.0
10 PreP	51M	LAD	1.7	27	2.2	1.4
PostP			2.0	21	3.1	2.4
PostS			2.3	15	4.1	3.6
11 PreP	41M	LAD	0.6	81	0.3	0.3
PostP			1.6	51	2.0	2.6
PostS			2.5	30	4.9	3.8
12 PreP	51F	LAD	0.9	61	0.6	1.3
PostP			2.1	25	3.5	4.3
PostS			2.0	26	3.1	2.9
13 PreP	69M	LAD	1.3	59	1.3	1.2
PostP			2.1	30	3.5	3.5
PostS			2.4	20	4.5	4.5
14 PreP	51F	Right	1.0	60	0.8	0.3
PostP			2.3	22	4.1	3.4
PostS			2.4	21	4.5	5.0
15 PreP	54M	LC	1.2	52	1.1	1.1
PostP			1.6	39	2.0	2.6
PostS			2.3	26	4.1	4.1
16 PreP	55F	LC	0.8	61	0.5	0.2
PostP			1.6	37	2.0	3.1
PostS			1.9	25	2.8	2.6
17 PreP	54F	LAD	1.2	61	1.1	1.2
PostP			1.7	35	2.3	4.5
PostS			2.1	20	3.5	3.9
18 PreP	52M	LAD	1.9	52	2.8	1.2
PostP			2.7	39	5.7	7.1
PostS			1.9	36	2.8	2.5
19 PreP	65M	LAD	1.1	63	0.9	0.0
PostP			2.3	17	4.1	1.6
PostS			2.6	13	5.3	2.9
Mean ± SD						
PreP			1.2 ± 0.4 *	58 ± 11 †	1.2 ± 0.7	0.9 ± 0.7
PostP			1.9 ± 0.3 *	32 ± 10 †	3.0 ± 1.0	3.5 ± 1.5
PostS			2.3 ± 0.3 ‡	22 ± 7 §	4.1 ± 1.2	3.9 ± 1.4

* P = 0.0000; † p = 0.0002.
LAD = left anterior descending artery; LC = left circumflex artery; PreP = before percutaneous transluminal coronary angioplasty; PostP = after percutaneous transluminal coronary angioplasty; PostS = after stenting; Right = right coronary artery; SD = standard deviation.

placed to cover the entire dilated arterial segment. Medications at the time of the initial angiogram were intravenous heparin, acetylsalicylic acid, dipyridamole, nitrates and calcium antagonists. Coronary angiograms were performed before and after angioplasty, and after stent implantation.

Description of the stent: In this trial, the endovascular prosthesis, Wallstent®, was provided by Medinvent SA, Lausanne. The method of implantation and description of this stent have been reported previously.[13,14] This stent is a self-expandable, stainless steel-woven mesh prosthesis that can be positioned in the coronary artery with the standard over-the-wire technique through an 8Fr or 9Fr guiding catheter. The device is constructed of sixteen 0.08-mm-wide wire filaments. It is constrained in an elongated configuration on a 1.57-mm-diameter delivery catheter, with the distal end covered by a removable plastic sleeve. As the sleeve is withdrawn, the constrained device returns to its original unconstrained larger diameter and becomes anchored against the vessel wall. Unconstrained stent diameter was selected to be 0.50 mm larger than the reference diameter of the stented vessel.

Quantitative coronary angiography: All cineangiograms were analyzed with the computer-assisted cardiovascular angiography analysis system, which has been discussed in detail previously.[15-18] The important steps will be briefly described. Any area sized 6.9 × 6.9 mm in a selected cineframe (overall dimensions 18 × 24 mm) encompasing the desired arterial segment can be digitized by a high-resolution CCD-camera with a resolution of 512 × 512 pixels and 8 bits of gray level. Vessel contours are determined automatically based on the weighted sum of the first and second derivative functions applied to the digitized brightness information along scanlines perpendicular to the local centerline directions of an arterial segment. A computer-derived estimation of the original arterial dimension at the site of the obstruction is used to define the interpolated reference diameter. This technique is based on a computer-derived estimation of the original diameter values over the analyzed region (assuming there was no disease



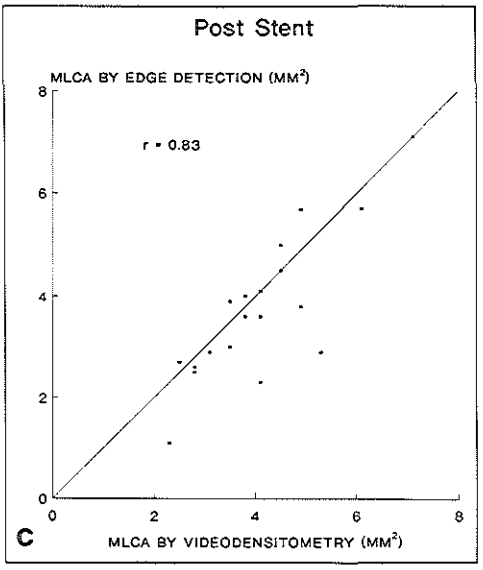


FIGURE 1. Individual data for minimal luminal cross-sectional area (MLCA) determined by edge detection and videodensitometry *(A)* before and *(B)* after percutaneous transluminal coronary angioplasty (PTCA) and *(C)* after stenting. *Diagonal line*, equal measurements by the 2 methods. Values above line were higher by edge detection and below line higher by densitometry.

present) according to the diameter function. The absolute diameter of the stenosis as well as the reference diameter are measured by the computer, which uses the known guiding catheter diameter as a calibration factor. All contour positions of the catheter and arterial segments are corrected for pincushion distortion. The minimal cross-sectional area of the narrowed segment and the interpolated percent area stenosis are then derived by assuming a circular model and comparing the observed stenosis dimensions to the reference values. The angiographic analysis was done using the view in which the arterial narrowing appeared the most severe and all interventions were performed.

Densitometric analysis: Densitometry is based on the approximate linear relation that exists between the optical density of a contrast-enhanced lumen and the absolute dimensions of the arterial segment. Constitution of the relation between the path length of the x-rays through the artery and the brightness values requires a detailed analysis of the complete x-ray/cine/video chain, including the film development process.

For the first part of the chain, from the x-ray tube to the output of the image intensifier, we use Lambert Beer's law for the x-ray absorption and apply certain models for the x-ray source and the image intensifier. From the output of the image intensifier up to the brightness values in the digital image, we use a simple linear transfer function. Details of this technique have been described elsewhere.[5,15-18]

The cross-sectional area of a vessel is then obtained as follows: When selecting a cineframe for the densitometric analysis, we ensure that the main axis of the segment is reasonably perpendicular to the incoming x-rays (i.e., a nonforeshortening view is chosen). Contours of the artery are detected by automated contour detection as previously described. From the measured diameters along the analyzed segment, the diameter data described above are derived. On each scanline perpendicular to the local centerline direction of the vessel, a profile of brightness values is measured. This profile is transformed into an absorption profile by means of a simple logarithmic transfer function. The background contribution is estimated by computing the linear regression line through the background points directly left and right of the detected contours. Subtraction of this background portion from the absorption profile within the arterial contours yields the net cross-sectional absorption profile. Integration of this function gives a measure for the cross-sectional area at the particular scanline. By repeating this procedure for all scanlines, the cross-sectional area function is obtained. A reference densitometric area is obtained following the same principles as previously described for the diameter measurements. It is clear that homogeneous mixing of the contrast agent and the blood must be assumed for the measurement to be correct. The complete procedure has been evaluated with the cinefilms of Plexiglas® models of coronary obstructions.[16]

To determine whether the physical properties of the stent itself interfere with the densitometric assessment, Wallstents® were placed inside known stenoses within perspex models and the minimal luminal cross-sectional area was calculated by densitometry. These cylindrical models, 5 mm in diameter at the ends and tapering to either 2 or 3 mm in the center, were filled with iopamidol (50 or 100% concentration) and angiographic studies were done at 75 kV to approximate the clinical setting. The calculated values for minimal luminal cross-sectional area were 0 to 12% higher in the stented models, compared with identical phantoms that did not contain stents.

Statistical analysis: The individual data for minimal luminal diameter and minimal luminal cross-sectional area by edge detection and densitometry, respectively, were used to calculate the mean value ± standard deviation (Table I). Analysis of variance was performed to compare the mean minimal luminal diameter before and after PTCA and after stenting and, if significant differences were found, 2-tailed t tests were applied. A value <0.05 was considered statistically significant.

To measure the strength of the relation between the 2 methods of analysis—edge detection and densitometry—in determining minimal luminal cross-sectional area, the product-moment correlation coefficient (r) and its 95% confidence intervals were calculated at the 3 distinct times of study. The agreement between the 2 measures was assessed by determining the mean and the standard deviation of the between-method difference, as

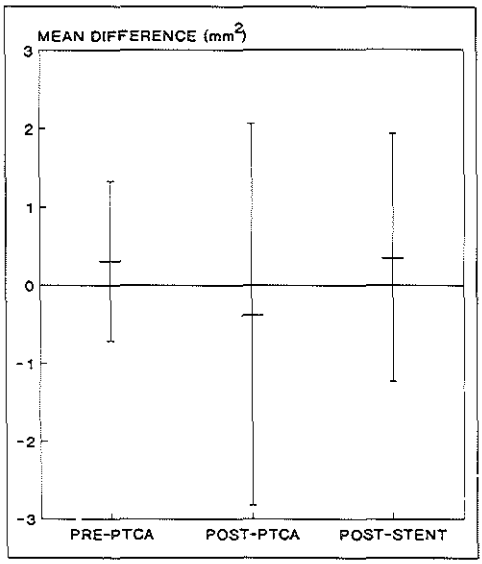


FIGURE 2. Mean difference between edge detection and densitometry and 95% confidence intervals before and after percutaneous transluminal coronary angioplasty (PTCA) and after stenting. Mean differences were slightly positive (0.31, 0.35 mm²) before PTCA and after stenting, respectively, and slightly negative (−0.38 mm²) after PTCA. The widest 95% confidence interval was in the analysis after PTCA, indicating the poorest association between the 2 methods, compared with the analysis before PTCA and after stenting.

suggested by Bland and Altman.[19] At each interval this was done by computing the sum of the individual differences between the 2 methods to determine the mean difference and the standard deviation.

RESULTS

The individual data obtained by contour detection and videodensitometric analysis are listed in Table I. There was an overall significant increase in the minimal luminal diameter and a decrease in percent diameter stenosis after angioplasty (1.2 ± 0.3 to 1.9 ± 0.3 mm and 58 ± 11 to 32 ± 10%, respectively) and after stenting (2.3 ± 0.3 mm, 22 ± 7%).

The correlation between edge detection and densitometry in the assessment of minimal luminal cross-sectional area before and after PTCA, and after stenting is shown in Figures 1A, 1B and 1C, respectively. Before angioplasty, correlation coefficient was 0.73 (95% confidence interval, 0.41 to 0.89), indicating a reasonably linear relation. However, this deteriorated after PTCA, resulting in a correlation coefficient of 0.59 (95% confidence interval, 0.15 to 0.83). However, linearity was significantly improved with the implantation of a coronary stent (correlation coefficient, 0.83; 95% confidence interval, 0.61 to 0.93).

The agreement between the 2 measures is illustrated in Figure 2. The determination of minimal luminal cross-sectional area was slightly higher by edge detection than by videodensitometry in the before PTCA and after stenting analyses (mean differences, 0.31 and 0.35 mm^2, respectively) and slightly lower after PTCA (mean difference, −0.38 mm^2). The variability as deter-

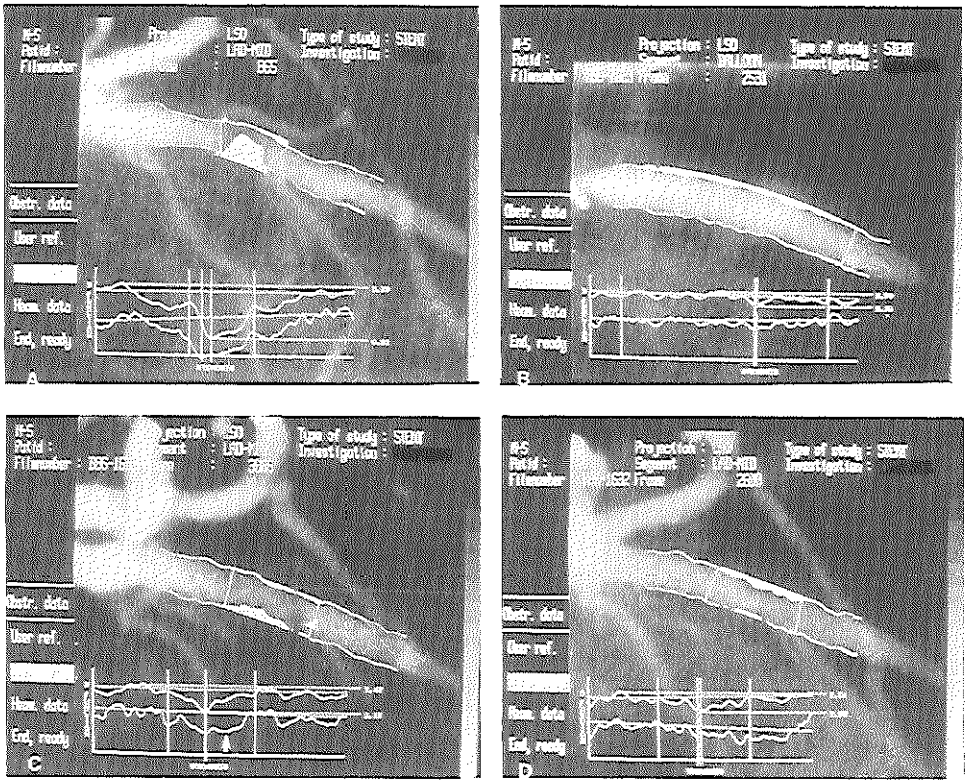


FIGURE 3. Edge contour and densitometric analysis of an obstruction in the left anterior descending (LAD) artery before percutaneous transluminal coronary angioplasty [PTCA] *(A)*, during balloon inflation *(B)*, after PTCA *(C)* and after stenting *(D)*. Graphs show the diagnostic diameter function *(upper curve)* and the densitometric area function *(lower curve)*. *Lower horizontal line* (0.81 mm in frame A) is the minimal luminal diameter. *Outside vertical lines* on the graph and the 2 *vertical lines* on the angiogram are lesion boundaries. *Inner vertical lines* on graph are the site in the lesion of minimal luminal diameter. In the angiogram before PTCA *(A)*, contour and densitometry curves are parallel. There was a marked improvement in the minimal luminal diameter of the lesion during balloon inflation *(B)*. After PTCA *(C)*, contour and densitometry curves diverge *(arrow)* at the site of an intraluminal haziness *(arrowhead)*. In the diameter function, there is a descending limb of the curve that reaches a nadir and immediately is followed by an ascending limb. However, the densitometry curve shows a descending limb followed by a plateau. After stenting *(D)*, the relation between the 2 curves is restored.

mined by the standard deviation of the differences between the 2 measurements was highest in the analysis after PTCA (1.22 mm^2), compared to before PTCA and after stenting (0.51 and 0.79 mm^2, respectively). An individual example is shown in Figure 3.

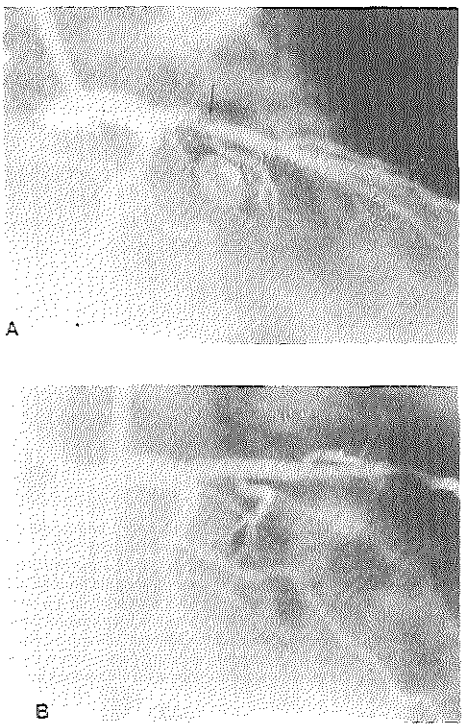
DISCUSSION

The ideal method by which to perform angiographic analysis after coronary interventions, including balloon angioplasty and stenting, remains debatable. Although densitometry is independent of geometric shape, its application is limited in the presence of branch vessels that may cause errors in the background correction technique and in situations where the x-ray beam is not perpendicular to the long axis of the vessel. Additional clinical factors that contribute to the inaccuracy of densitometry include x-ray scatter, light scatter within the image intensifier (veiling glare) and beam hardening of the polychromatic x-ray flux because of iodine and tissue thickness. Discrepancies between edge detection and densitometry are most likely to occur when the shape of the vessel wall at the level of the lesion deviates furthest from a circular configuration, because this is a basic assumption in the calculation of minimal luminal cross-sectional area by edge detection.

This study illustrates several important points. First, we have shown that a relation exists between the 2 measurements at all stages of the procedure, but the strength of this relation, based on the magnitude of the correlation coefficient, deteriorates after PTCA and then improves after stenting. Furthermore, although mean differences between the 2 methods were small in all analyses, the greatest variability and thus the poorest agreement occurred in the analysis after PTCA. The before and after PTCA results are in accordance with earlier observations by our group.[5] At that time we suggested that measurement of cross-sectional area from a single view is inaccurate. Subsequent studies by Tobis et al[11] comparing edge detection in 2 orthogonal views and by Lesperance et al[12] comparing single versus the mean of multiple views have shown similar and high correlations both before and after PTCA. However, use of the correlation coefficient alone is not an adequate measure of agreement between 2 measurement techniques for several statistical reasons.[19,20] Determination of the mean and standard deviation of the between-method differences should be included in the analysis.

Two factors probably contributed to the improved agreement after stent implantation. Vessel contours appeared more regular and smooth and in some cases intimal flaps appeared to be tacked back by the scaffolding property of this stent. However, even more important, the self-expanding property of this stent not only additionally dilated the vessel, but also probably remodeled the stented segment into a more circular geometry. This has previously been shown in vivo after the implantation of coronary stents in animals and in some human coronary vessels (Figure 4, A, B and C).

A potential limitation of densitometry in the analysis after stenting may be a spuriously high determination of minimal luminal cross-sectional area (up to 12% in the



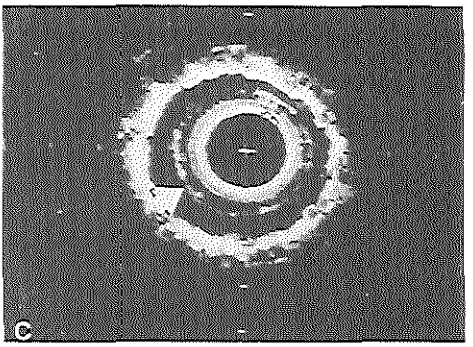


FIGURE 4. ***A,*** **angiogram of left anterior descending artery stenosis after dissection** ***(arrow)*** **during percutaneous transluminal coronary angioplasty.** ***B,*** **angiographic appearance of left anterior descending artery lesion after stenting showing smooth contour.** ***C,*** **in vitro intravascular ultrasound examination of this vessel 24 hours after stenting (patient died from intracerebral hemorrhage 12 hours after stenting). The inner circle is due to intravascular probe. The outer echodense pattern is due to stent wires** ***(large arrow).*** **The lumen** ***(small open arrow)*** **is the echo-free space inside the stent. The stent effectively tacked back the dissection and restored the circular configuration of the vessel (courtesy of Dr. Bernardino Tucillo).**

phantom studies) because of interference from the stent itself. This is probably related to the composition of the stent, surface area or additional factors, such as increased scatter in the stenotic section because of the stent. Although the mean differences in minimal luminal cross-sectional area between the edge detection and densitometry were small, the negative mean difference in the analysis after stenting (i.e., larger values by densitometry) in contrast to the positive mean difference after PTCA can be partly explained by this contribution of the stent to the densitometrically determined values. The effect of other currently available stents should be separately assessed and considered in angiographic analyses using densitometry.

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Chapter 5

Angiographic follow-up after placement of a self-expanding coronary stent

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ANGIOGRAPHIC FOLLOW-UP AFTER PLACEMENT OF A SELF-EXPANDING CORONARY-ARTERY STENT

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Abstract *Background.* The placement of stents in coronary arteries after coronary angioplasty has been investigated as a way of treating abrupt coronary-artery occlusion related to the angioplasty and of reducing the late intimal hyperplasia responsible for gradual restenosis of the dilated lesion.

Methods. From March 1986 to January 1988, we implanted 117 self-expanding, stainless-steel endovascular stents (Wallstent) in the native coronary arteries (94 stents) or saphenous-vein bypass grafts (23 stents) of 105 patients. Angiograms were obtained immediately before and after placement of the stent and at follow-up at least one month later (unless symptoms required angiography sooner). The mortality after one year was 7.6 percent (8 patients). Follow-up angiograms (after a mean [±SD] of 5.7±4.4 months) were obtained in 95 patients with 105 stents and were analyzed quantitatively by a computer-assisted system of cardiovascular angiographic analysis. The 10 patients without follow-up angiograms included 4 who died.

Results. Complete occlusion occurred in 27 stents in 25 patients (24 percent); 21 occlusions were documented within the first 14 days after implantation. Overall, immediately after placement of the stent there was a significant increase in the minimal luminal diameter and a significant decrease in the percentage of the diameter with stenosis (changing from a mean [±SD] of 1.88±0.43 to 2.48±0.51 mm and from 37±12 to 21±10 percent, respectively; $P<0.0001$). Later, however, there was a significant decrease in the minimal luminal diameter and a significant increase in the stenosis of the segment with the stent (1.68±1.78 mm and 48±34 percent at follow-up). Significant restenosis, as indicated by a reduction of 0.72 mm in the minimal luminal diameter or by an increase in the percentage of stenosis to ≥50 percent, occurred in 32 percent and 14 percent of patent stents, respectively.

Conclusions. Early occlusion remains an important limitation of this coronary-artery stent. Even when the early effects are beneficial, there are frequently late occlusions or restenosis. The place of this form of treatment for coronary artery disease remains to be determined. (N Engl J Med 1991; 324:13-7.)

TWO major limitations of coronary angioplasty are acute occlusion and late restenosis. The concept of implanting an endoluminal stent in the coronary arteries after balloon dilation to circumvent these problems was first suggested in 1964.[1] This procedure was successfully performed in patients in 1986.[2] In May 1988, the five European centers testing this device agreed to set up a core laboratory for quantitative angiographic analysis to assess the results objectively. The early follow-up results reported by the core laboratory showed that immediately after stent implantation, there was an additional increase in the minimal luminal diameter of the vessel and a decrease in the percentage of stenosis of the diameter.[3] However, after three months slight but diffuse narrowing was observed in the artery containing the stent.[4] In the present study, we have focused on the results of long-term angiographic follow-up of the initial 117 stent implantations.

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METHODS

Study Patients

One hundred five patients gave informed consent and were enrolled at participating study centers between March 1986 and January 1988. The study protocol was approved by the ethics committees of the individual hospitals. The clinical characteristics of the patients are shown in Table 1. Ninety-five patients received one stent, and 10 received more than one (Table 1). Seven of the 10 patients who received multiple stents required two overlapping ("telescoping") stents to cover long lesions adequately, and the other 3 required stents in multiple vessels or in different locations in the same vessel. The sites of stent placement are shown in Table 1. Seventy-one stents were implanted after redilation of a restenosis, 14 were placed as an emergency procedure during an angioplasty complicated by acute occlusion, 5 were placed after angioplasty for chronic occlusion, and 27 were placed as an adjunct procedure to primary percutaneous transluminal coronary angioplasty (PTCA). Some of the patients who received stents for bypass grafting or abrupt closure have been included in previous reports.[5,6]

In this trial, the endovascular prosthesis Wallstent (Medinvent, Lausanne, Switzerland) was used. The method of implantation and a description of this stent have been previously reported.[2] This stent is a self-expanding, stainless-steel, woven-mesh prosthesis that can be positioned in the coronary artery with an 8-French or 9-French guiding catheter, according to the standard over-the-wire technique. The device is constructed of 16 wire filaments, each 0.08 mm wide. It is constrained in an elongated configuration on a delivery catheter 1.57 mm in diameter; the distal end of the prosthesis is covered by a removable plastic sleeve. As the sleeve is withdrawn, the constrained device returns to its original, larger diameter and becomes anchored against the vessel wall. The diameter of the prosthesis ranges from 2.5 to 6 mm when the stent is unconstrained.

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Table 1. Clinical Characteristics of the Study Patients.

No. of patients	105
Age (yr)*	57±9
Sex (M/F)	91/14
No. of stents	117
Site of stent implantation (no. of stents)	
Left anterior descending artery	62
Circumflex artery	6
Right coronary artery	26
Bypass graft	23
Indication for implantation (no. of stents)	
Restenosis	71
PTCA with acute occlusion	14
PTCA as adjunct procedure	27
Chronic occlusion	5
Time to angiographic follow-up (mo)*	
All patients	5.7±4.4
Patients with patent stents	7.2±3.6
No. of stents per patient (no. of patients)	
One	95
Two	8
Three	2

*Means ±SD.

We selected a diameter 0.50 mm larger than the reference diameter of the stenosed vessel.

Anticoagulation regimens evolved throughout the study period, and different protocols were used at the various centers. In the first 23 of the 32 patients treated in Toulouse, heparin was administered subcutaneously three times a day to maintain the activated cephalin–kaolin time (an index of coagulation status) at twice the control value, starting three to five days before the procedure and continuing for six weeks afterward. During the procedure, the 7th through the 32nd patients received an additional 10,000 units of heparin intravenously and 20,000 to 50,000 units of streptokinase by intracoronary infusion. Aspirin (100 mg) and dipyridamole (300 mg) were given daily by mouth, starting 24 hours before the procedure. The 24th through the 32nd patients treated in Toulouse received a vitamin K antagonist (warfarin or acenocoumarol) by mouth, started on the day of the procedure and continued for three to six months. The subcutaneous heparin injections were stopped after a therapeutic level of oral anticoagulant was reached (International Normalized Ratio, ≥2.3). In the other centers, aspirin (1 g orally) was started one day before the procedure. Heparin (10,000 to 15,000 units) and urokinase (100,000 units by intracoronary infusion) were administered during the procedure. Heparin was given intravenously, and then the vitamin K antagonist by mouth for three to six months as described above. Aspirin (initially 1 g daily and later 100 mg daily), dipyridamole (300 to 450 mg daily), and in some patients sulfinpyrazone (400 mg daily) were also administered. The first four patients treated in Rotterdam did not receive aspirin.

Quantitative Coronary Arteriography

All cineangiograms were analyzed at the core laboratory in Rotterdam by means of a computer-assisted cardiovascular-angiography analysis system, discussed in detail elsewhere.[7,8] The important steps will be briefly described. Selected areas of the cine frame encompassing the desired arterial segment were optically magnified, displayed in a video format, and then digitally converted. Vessel contour was determined automatically on the basis of the weighted sum of the first and second derivative functions applied to the digitized information on brightness. A computer-derived estimation of the original dimensions of the artery at the site of the obstruction was used to determine interpolated reference values for arterial diameter and area. The absolute diameter of the segment with stenosis as well as the reference diameter was measured by the computer, which used the diameter of the guiding catheter as a calibration factor, after correcting for pincushion distortion. The interpolated percentage of stenosis of the narrowed segment was then derived by assuming a circular model and comparing the observed value for stenosis with the reference value. The minimal luminal diameter of each segment immediately proximal and distal to the stent was also measured. The angiographic analysis was performed before and after angioplasty, immediately after stent implantation, and at long-term follow-up evaluation in all patients, with the use of the average of multiple matched views with orthogonal projections whenever possible.

Restenosis

Two different sets of criteria were applied to determine the rate of restenosis. We have found a reduction of 0.72 mm or more in the minimal luminal diameter to be a reliable indicator of angiographic progression of vessel narrowing.[7-9] This value takes into account the limitations of coronary angiographic measurements and represents twice the long-term variability of repeat measurements of a coronary-artery obstruction with the cardiovascular-angiography analysis system. The other criterion for restenosis was an increase in the percentage of stenosis from less than 50 percent after stent implantation to 50 percent or more at follow-up evaluation. This criterion was selected since common clinical practice has continued to express lesion severity as a percentage of stenosis.

Statistical Analysis

Values obtained by quantitative angiographic analysis are expressed as means ±SD. The means for each angiographic variable before PTCA, after PTCA, immediately after placement of the stent, and at follow-up were compared by analysis of variance. If significant differences were found, two-tailed t-tests were applied to paired data. A statistical probability of less than 0.05 was considered to indicate significance.

The results of angiographic and clinical follow-up were expressed in a life-table format according to the Kaplan–Meier method.[10] Stent occlusions, cardiac deaths (which were assumed to be due to occlusion, for statistical purposes), and restenosis as defined by the two criteria were considered angiographic end points. The following events were considered clinical end points: death, myocardial infarction, bypass surgery, and nonsurgical revascularization (PTCA or atherectomy). The life table was constructed according to the initial clinical event.

Results

The overall mortality after one year was 7.6 percent (eight deaths) (Table 2). The mean (±SD) period of angiographic follow-up was 5.7±4.4 months in all patients and 7.2±3.6 months in patients whose stents were patent at follow-up. Angiographic follow-up (Fig. 1) was performed in 95 patients (90 percent) with 105 stents (90 percent); 78 stents were patent, and 27 were occluded (Table 3). Angiographic follow-up could not be obtained in 10 patients for the following reasons: 4 patients died, 4 refused follow-up angiography, and 2 had follow-up angiograms that were technically inadequate for analysis (but did not show total occlusion). Twenty angiograms were obtained during the first month after stent implantation; all were obtained because clinical symptoms had occurred, and all showed occlusions. Angiograms obtained after the first month were part of the routine follow-up evaluation; all showed patent stents except in five patients with stent occlusions. Overall, the minimal luminal diameter increased from 1.21±0.56 mm to 1.88±0.43 mm after PTCA and then further, to 2.48±0.51 mm, immediately after stent implantation ($P<0.0001$) because of the intrinsic dilator function of

the device (Table 4). At follow-up the diameter was found to have decreased to 1.68±1.20 mm. The percentage of stenosis changed similarly, with an initial decrease from 61±14 to 37±12 percent after angioplasty and an additional decrease to 21±10 percent immediately after stent placement ($P<0.0001$). However, at follow-up the percentage of stenosis had increased to 48±34 ($P<0.0001$). When only patent stents were included in the analysis of late follow-up, the minimal luminal diameter and the percentage of stenosis were 2.26±0.78 mm and 30±17 percent, respectively. A small, nonsignificant increase occurred in the reference diameter after stent placement (from 3.15±0.54 to 3.22±0.79 mm). During the study, no significant change was seen in the minimal luminal diameter of the proximal or distal segments adjacent to the stent.

The incidence of restenosis (Fig. 2) depended on the definition of stenosis (Fig. 3). When a change of ≥0.72 mm in minimal luminal diameter was used as a criterion, restenosis was observed within the patent stent in 17 patients (19 stents), in the proximal segment adjacent to the stent in 3 patients, in the segment immediately distal to the stent in 2 patients, and in both proximal and distal regions in 1 patient. Therefore, the total rate of restenosis was 32 percent among stents and 33 percent among patients. At follow-up the percentage of stenosis had increased to ≥50 percent within 10 stents (13 percent) in 9 patients (13 percent) and in the segment proximal to the stent in 1 stent in 1 patient, for a total rate of 14 percent. After one year of clinical follow-up, two of the patients with restenosis underwent repeat balloon angioplasty, one patient (two stents) underwent atherectomy, performed within the narrowed stent, and six patients underwent coronary bypass surgery. Death or myocardial infarction did not occur in any of these nine patients.

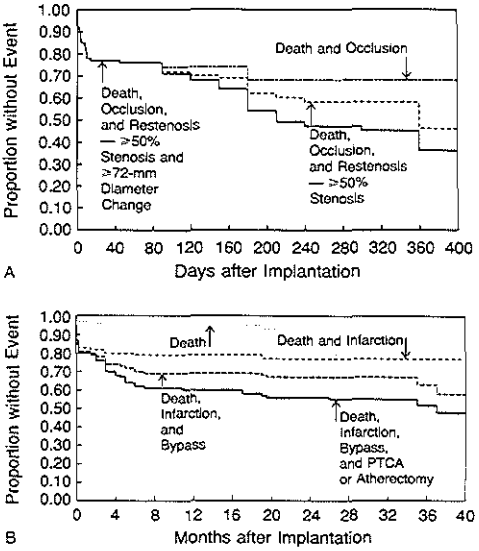


Figure 1. Angiographic and Clinical Follow-up in 95 Patients Who Received 105 Stents.

Occlusion of the stent, cardiac death, and restenosis as determined by either or both of the criteria used (≥50 percent stenosis of the vessel and a change of ≥72 mm in the minimal luminal diameter) were considered angiographic end points (Panel A). Death, myocardial infarction, bypass surgery, and PTCA or atherectomy were considered clinical end points (Panel B).

Table 2. Deaths after Stent Implantation.

Patient No.	Time after Implantation	Cause of Death
1	<24 hr	Stent occlusion after vessel closure during PTCA
2	48 hr	Sudden death
3	2 days	Stent occlusion after 24 hr, followed by emergency bypass procedure
4	8 days	Stent occlusion during implantation, myocardial infarction, shock
5	11 days	Sudden death
6	1½ mo	Sudden death
7	2½ mo	Surgery for new lesion of left main artery, after bypass procedure
8	6 mo	Chronic congestive heart failure

Discussion

The data from the six European centers at which the coronary-artery stent described above was used show a stent-occlusion rate of 24 percent. The anticoagulation regimens and methods for selecting patients differed among the centers, which may explain some of the variability in the occlusion rates between centers. The highest occlusion rate (39 percent) was observed at the Toulouse center, where the initial patients were treated with long-term subcutaneous heparin after placement of the stent, instead of a vitamin K antagonist. The clinical factors that contributed to the occlusions could be identified in 11 patients — i.e., disorders of the coronary artery that are associated with thrombosis (unstable angina, recent myocardial infarction, and chronic occlusion) in 5 patients, technical problems in stent placement in 3 patients, interruption of anticoagulation because of

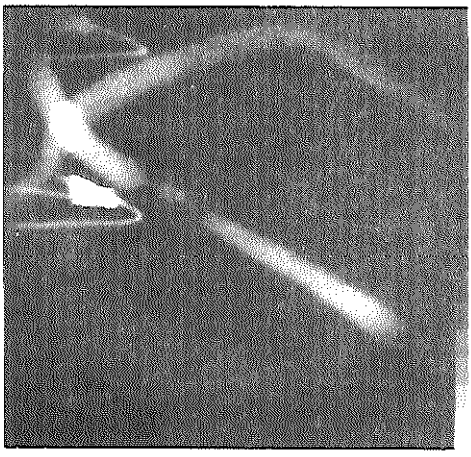
Table 3. Findings at Angiographic Follow-up.

Finding	No. of Stents (N = 117)	No. of Patients (N = 105)
	number (percent)	
Patent stent	78 (67)	70 (67)
Occluded stent	27 (23)	25 (24)
No follow-up angiography		
Death	6 (5)	4 (4)
Refusal	4 (3)	4 (4)
Inadequate study	2 (2)	2 (2)

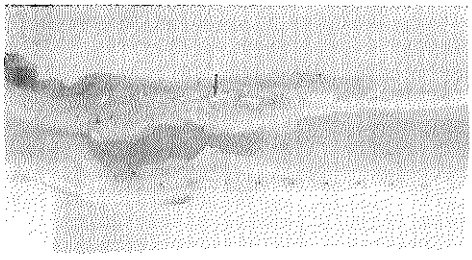
Table 4. Findings at Quantitative Angiography.*

VARIABLE	BEFORE PTCA	AFTER PTCA	AFTER STENT IMPLANTATION	AT FOLLOW-UP	
				PATENT STENTS	ALL STENTS
Minimal luminal diameter (mm)	1.21±0.56	1.88±0.43	2.48±0.51	2.26±0.78	1.68±1.20
Stenosis (%)	61±14	37±12	21±10	30±17	48±34
		P<0.0001	P<0.0001	P<0.0001	
			P<0.002		

*Values (means ±SD) were compared by analysis of variance. If significant differences were found, two-tailed t-tests were applied to pairs of data. A probability of 0.05 was considered to indicate statistical significance.



A



B

Figure 2. Restenosis Six Months after Implantation of a Stent in a Bypass Graft.

In the follow-up angiogram (Panel A), the outline of the stent appears slighly radiopaque and hyperplasia has resulted in a complex narrowing within the vessel segment containing the stent.

In the gross specimen (Panel B) of the surgically retrieved bypass graft containing the segment shown in Panel A, the longitudinal cross section of the vessel shows the stent filaments (arrow) protruding from the wall. The striking similarity between the angiographic contours of the vessel and its actual appearance is evident.

bleeding problems in 2 patients, and hemodynamic compromise before placement of the stent in 1 patient with cardiogenic shock. In view of the early experience with stent occlusion, the investigators agreed to avoid placing stents in patients with acute coronary artery disorders and chronic occlusions or in patients with poor distal runoff (vessels with collateral flow, small vessels less than 3 mm in diameter, or vessels supplying akinetic or severely hypokinetic myocardium). In addition, four patients with six stents died before undergoing angiographic follow-up. Some of these deaths were sudden, suggesting possible stent occlusion. It was difficult to determine whether late occlusion (after 14 days) was superimposed on marked restenosis. Therefore, the rates of occlusion and restenosis may have been underestimated.

The patients in this study underwent two serial interventions, balloon dilation and then stent implantation. Quantitative coronary angiography showed that the initial effect of angioplasty in these patients was similar to that observed in previous angiographic studies,[8,11] and moreover, the result immediately after placement of the stent was markedly improved. However, the minimal luminal diameter in the entire study group at follow-up (including patients known to have occlusions) was 1.68 mm, which is comparable but not superior to values previously documented in late follow-up studies of coronary balloon angioplasty (1.69 to 1.82 mm).[8,11] The rate of restenosis in patent stents, when based on a change of ≥0.72 mm in the minimal luminal diameter, was 23 percent among segments within the stent and 8 percent among segments adjacent to the stent, for a total rate of 31 percent. When the alternative criterion, ≥50 percent stenosis of the luminal diameter, was used, the rate of restenosis was 13 percent among segments within the stent and 1 percent among segments adjacent to the stent. Two previous studies that used similar quantitative methods for late follow-up evaluation after coronary balloon angioplasty have been published. In one study,[8] the restenosis rate for angiograms obtained at four months was 25.5 percent when the criterion of a change of 0.72 mm was used, and 13.2 percent when the criterion of 50 percent stenosis was used. In the other

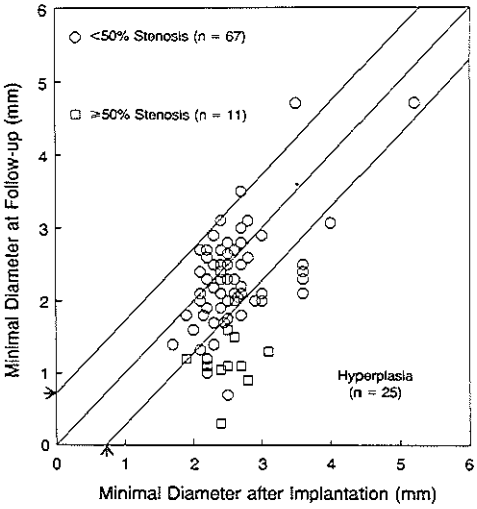


Figure 3. Change in the Minimal Luminal Diameter of 78 Patent Stents between Stent Implantation and Angiographic Follow-up. The diameter of each segment immediately after implantation is plotted against the diameter at follow-up. The lines on each side of the identity line (diagonal) represent the limits of long-term variability of repeat measurements (a change of ≥0.72 mm [arrows]). The symbols below the right-hand line represent stents with involvement by severe hyperplasia.

study,[12] when the criterion of 50 percent stenosis was used, the restenosis rate was 37 percent for angiograms obtained four to seven months after angioplasty.

Studies in animals have confirmed that fibrointimal hyperplasia may develop in arterial segments containing stents. Within one week after stents (Wallstent) were implanted in normal porcine arteries, the prostheses became completely covered by endothelium and the vessel lumen had diffuse narrowing, varying in thickness from 60 to 125 μm.[13,14] By six months, the thickness of the neointima increased from 50 to 400 μm,[15] corresponding to a decrease of 0.1 to 0.8 mm in the vessel diameter.

Early thrombotic occlusion remains a serious clinical problem with this prosthesis despite anticoagulation. It remains to be determined whether increased experience of operators, changes in the anticoagulation regimen, or selection of patients will circumvent this limitation. New biologic coatings that may make the stent less thrombogenic are currently under investigation. Angiographically detectable narrowing, probably due to fibrointimal hyperplasia, occurs to a marked degree in patients whose stents are patent at late follow-up. Although six months is assumed to be the time frame for the development of restenosis after angioplasty, this may not be true of stent implantation. The clinical indications for the use of an endovascular prosthesis remain unclear. Controlled clinical trials are imperative to determine whether such devices can decrease the rate of restenosis among patients who have undergone PTCA and whether they can be of any benefit in particular clinical situations or subgroups of patients.

We are indebted to Dr. Jan Tyssen, Dr. Roger Thalmann, Marie-Angèle Morel, and Eline Montauban van Swijndregt for assistance.

Appendix: Participating Centers and Collaborators

Catheterization Laboratory for Clinical and Experimental Image Processing, Thoraxcenter, Rotterdam, the Netherlands: B.H. Strauss, M.D., K.J. Beatt, M.B., B.S., M. v.d. Brand, M.D., P.J. de Feyter, M.D., H. Suryapranata, M.D., I.K. de Scheerder, M.D., J.R.T.C. Roelandt, M.D., and P.W. Serruys, M.D.; Department of Cardiology, Hôpital Cardiologique, Lille, France: M.E. Bertrand, M.D., and J.M. Lablanche, M.D.; Department of Clinical Measurement, National Heart Institute, London: A.F. Rickards, M.D., and U. Sigwart, M.D.; Department of Clinical and Experimental Cardiology, Centre Hospitalier Regional Universitaire, Rangeuil, Toulouse, France: J.P. Bounhoure, M.D., A. Courtault, M.D., F. Joffre, M.D., J. Puel, M.D., and H. Rousseau, M.D.; Division of Cardiology, Department of Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland: P. Vogt, M.D., J.-J. Goy, M.D., J.-C. Stauffer, M.D., U. Kaufmann, M.D., L. Kappenberger, M.D., and P. Urban, M.D.

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Chapter 6

A relative risk analysis of the angiographic predictors of restenosis in the coronary Wallstent

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Abstract

Background: Late angiographic narrowing has been observed following coronary implantation of the Wallstent®. To identify the angiographic variables that predict restenosis within the stented segment, a retrospective study of data from the European Wallstent core laboratory was performed.

Methods and Results: Follow-up angiograms (excluding patients with in hospital occlusions) were analyzed for 214 lesions in 176 patients (78% restudy rate). The incidence of restenosis within the stented segment was 35% by lesion and 35% by patient for Criterion 1 (≥0.72 mm loss in minimal luminal diameter) and 24% by lesion and 24% by patient for Criterion 2 (diameter stenosis ≥50% at follow-up). The association between 16 variables and restenosis was determined by a relative risk ratio assessment. Variables with significant risk ratios for restenosis with Criterion 1 were use of multiple stents/lesion (relative risk 1.56, 95% confidence interval (CI) 1.08-2.25) and oversized (unconstrained stent diameter exceeding reference diameter >0.7 mm) stents (relative risk 1.64, 95% CI 1.10-2.45); and for Criterion 2, oversizing by >0.70 mm (RR 1.93, 95% CI 1.13-3.31), bypass grafts (relative risk 1.62, 95% CI 0.98-2.66), use of multiple stents/lesion (relative risk 1.61, 95% CI 0.97-2.67) and residual diameter stenosis > 20% post stenting (relative risk 1.51, 95% CI 0.91-2.50).

Conclusions:It is concluded that several angiographic variables are significantly associated with late angiographic narrowing after stenting in the coronary arteries. We suggest that stent operators avoid excessive oversizing in the selection of stent diameter and the use of multiple stents per lesion to lessen the risk of late restenosis.

Key Words: Stents, Coronary Angioplasty, Restenosis, Coronary Artery Disease

INTRODUCTION

The implantation of stents in coronary arteries or saphenous vein bypass grafts as an adjunct or alternative to percutaneous transluminal coronary angioplasty (PTCA) was initially proposed to prevent late restenosis (1). Since March 1986, the coronary Wallstent[R] has been the most intensively studied endovascular prosthesis in Europe. As a result of cooperation among the six participating European centers, a central core laboratory was set up in Rotterdam to objectively assess the follow up of stents with quantitative coronary angiography. In our previous report on the initial 117 stents implanted in 105 patients, we observed that late angiographic narrowing occurs in a significant number of patients (2). To further characterize the factors associated with angiographic restenosis within the stented segment, we retrospectively studied the predictive ability of several angiographic variables, based on our experience of 214 separate lesions implanted with the coronary Wallstent in 176 patients. Since the core laboratory was set up only as an angiographic data bank, detailed clinical data was not available for this analysis.

METHODS

Study Patients

Two hundred and sixty-five patients were enrolled after obtaining informed consent between March 1986 and March 1990 at the six participating centers. The study group consisted of 222 men and 43 women with a mean age of 58 ± 11 years. Sixty-two percent of the stents were implanted in native vessels and 38% were placed in bypass grafts. In the overall group, angiographic follow up was obtained in 218 patients (82%). However in-hospital occlusions (40 patients, 41 lesions) were excluded from this study since these were definite thrombotic events and the objective of this study was late angiographic narrowing. Follow-up angiograms were quantitatively analyzed in 176 patients (78%) of the 225 patients who were discharged from hospital without known occlusion (Figure 1 and 2). Patients could not be restudied for the following reasons: death (n=10), early bypass surgery as per protocol for the "bail-out" indication at one institution or due to contraindication to anticoagulation (n=11), angiograms that were technically inadequate for quantitative analysis (n=3) or refusal for restudy (n=25). The study group consisted of 176 patients who had a total of 259 stents implanted in 214 lesions. The mean length of angiographic follow-up in the study group was 6.6 ± 4.8 months.

In this trial, the endovascular prosthesis, Wallstent[R], was provided by Medinvent SA, Lausanne. The method of implantation and description of this stent has previously been reported (1,2). This stent is a self-expandable stainless steel woven mesh prosthesis that can be positioned in the coronary artery using standard over-the-wire technique through a 8F or 9F guiding catheter. The device is constructed of 16 wire filaments, each 0.08 mm wide.

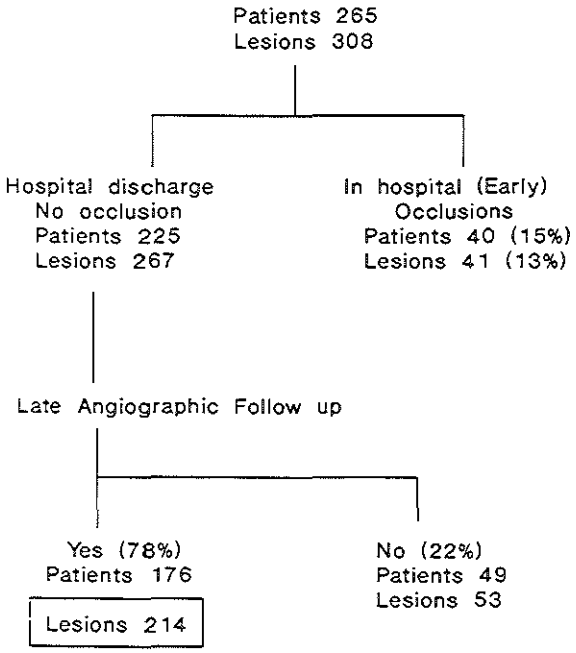

Figure 1. *Flow diagram showing the angiographic follow-up in 265 stented lesions. In hospital (early) occlusions occurred in 40 patients (15%). In the remaining 225 patients that were discharged from hospital without known stent occlusion, 176 patients (78%) with 214 stented lesions had quantitative angiographic follow-up.*

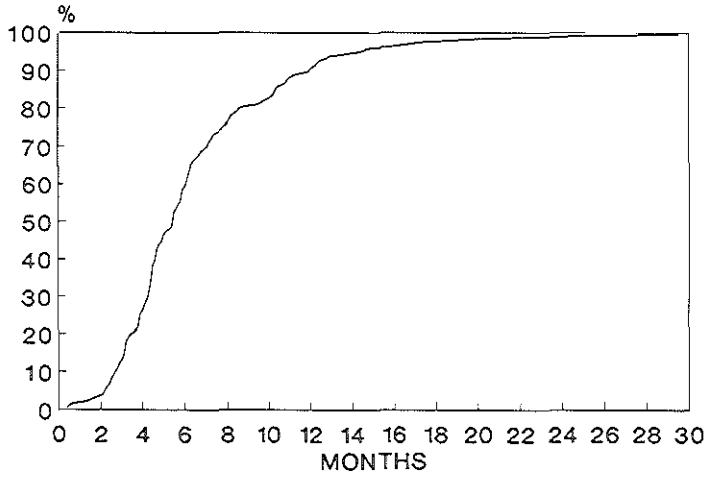

Figure 2. *Timing of late angiographic follow-up after stent implantation. In this cumulative curve, the interval (in months) between date of implantation and final angiographic follow-up is shown for the study group.*

It is constrained in an elongated configuration on a 1.57 mm diameter delivery catheter with the distal end covered by a removable plastic sleeve. As the sleeve is withdrawn, the constrained device returns to its original unconstrained larger diameter and becomes anchored against the vessel wall. Unconstrained stent diameter ranged from 2.5-6 mm and was selected to be 0.50 mm larger than the stented vessel based on a visual estimate of the pre stent angiogram by the investigator. In an effort to alleviate the problem of acute thrombosis, the stent design was changed in April 1989 with the introduction of a polymer coated stent (Biogold®) for certain stent sizes. By August 1989, all manufactured stents contained this particular polymer coating.

Quantitative Coronary Arteriography

All cineangiograms were analyzed at the core laboratory in Rotterdam using the computer assisted cardiovascular angiography analysis system (CAAS) which has previously been discussed in detail (3,4). The important steps will be briefly described. Selected areas of the cineframe encompassing the desired arterial segment (from side branch to side branch) are optically magnified, displayed in a video format and then digitally converted. Vessel contour is determined automatically based on the weighted sum of the first and second derivative functions applied to the digitized brightness information. A computer-derived estimation of the original arterial dimension at the site of the obstruction is used to define interpolated reference diameter and area. The absolute diameter of the stenosis as well as the reference diameter are measured by the computer which uses the known guiding catheter diameter as a calibration factor, after correction for pincushion distortion. The percentage diameter of the narrowed segment is derived by comparing the observed stenosis dimensions to the reference values. The length of the lesion (mm) is determined from the diameter function on the basis of a curvature analysis. Using the reconstructed borders of the vessel, the computer can calculate a symmetry coefficient for the stenosis. Differences in distance between the actual and reconstructed vessel contours on both sides of the lesion are measured. Symmetry is determined by the ratio of these two differences with the largest distance between actual and reconstructed contours becoming the denominator. Values for symmetry range from 0 for extreme eccentricity to 1 for maximal symmetry (that is, equal distance on both sides between reconstructed and actual contours). The angiographic analysis was done pre and post angioplasty, immediately post stent implantation and at long term follow-up in all patients using the average of multiple matched views with orthogonal projections wherever possible.

Restenosis

The restenosis rate was determined according to two criteria. We have found a loss in minimal luminal diameter (MLD) of 0.72 mm or more to be a reliable indicator of angiographic progression of vessel narrowing (3,4). This value takes into account the limitations of coronary angiographic measurements and represents twice the long term variability (ie.the 95% confidence interval) for repeat measurements of a coronary obstruction using CAAS. The second criterion for restenosis was an increase in diameter

stenosis (DS) from less than 50% after stent implantation to greater than 50% at follow-up. This criterion was selected since common clinical practice continues to assess lesion severity by a percentage stenosis.

Angiographic Variables

Based on the quantitative angiographic data, multiple variables were identified and recorded for each lesion. These variables, either discrete (two or three distinct responses) or continuous (a range of responses), were grouped according to lesion, stent or procedural factors (Tables 1,2). These particular variables were of a priori clinical interest on the basis of previously published PTCA and stent reports (5-11).

Statistical Methods

A relative risk analysis was performed for the aforementioned discrete and continuous variables (12). The continuous variables were dichotomized for the risk ratio analysis. To avoid arbitrary subdivision of data in continuous variables, cutpoints were derived by dividing the data into two groups, each containing roughly 50% of the total population. This method of subdivision has the advantage of being consistent for all variables and thus avoids any bias in selection of subgroups which might be undertaken to emphasize a particular point. The incidence of restenosis in the two groups was compared using a relative risk analysis. A relative risk of 1 for a particular variable implies that the presence of that variable poses no additional risk for restenosis; relative risks greater than 1 or less than 1 imply additional or a reduction in risk, respectively. For example a relative risk of 2 for a particular parameter implies that the presence of that factor increases the likelihood of restenosis by a factor of two. The 95% confidence intervals were calculated to describe the statistical certainty. Statistical significance was defined as $p < 0.05$. and was determined using the Pearson Chisquare (BMDP statistical software, University of California, Berkeley, California, 1990).

RESULTS

The incidence of "restenosis" depended upon the definition. Using a criterion of a change in minimal luminal diameter of $\geq$ 0.72 mm, restenosis occurred within the stented segment in 35% of lesions and 35% of patients. An increase in percent diameter stenosis to $\geq$ 50% at follow-up was seen in 24% of lesions and 24% of patients.

The relative risk and 95% confidence intervals for each variable using either of the two criterion for restenosis are shown in Figure 3. The variables with statistically significant associations with restenosis using the 0.72 mm criterion were multiple stents and oversizing the stent (unconstrained diameter) with respect to the reference diameter by more than 0.70 mm which had relative risk ratios (RR) (and 95% confidence intervals (CI)) of 1.56 (1.08-2.25) and 1.64 (1.10-2.45) respectively. The second criterion,

≥50% diameter stenosis at follow up, was associated with oversizing by >0.70 mm (RR 1.93, 95% CI 1.13-3.31), bypass grafts (RR 1.62, 95% CI 0.98-2.66), multiple stents/lesion (RR 1.61, 95% CI 0.97-2.67) and residual diameter stenosis > 20% post stenting (RR 1.51, 95% CI 0.91-2.50). The actual restenosis rates for these variables are included in Tables 3 and 4.

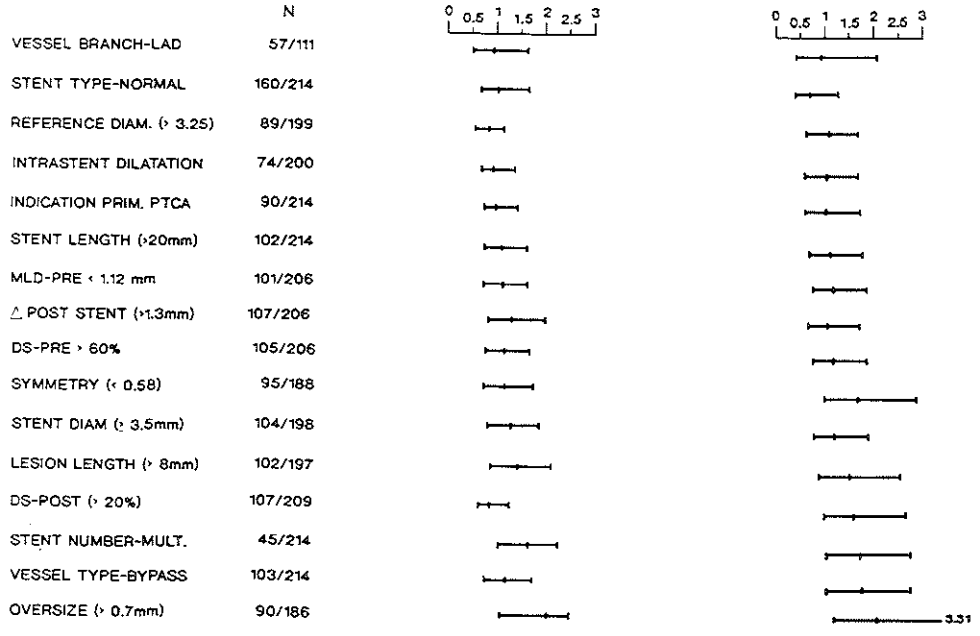

Figure 3. *Relative risk ratios (with 95% confidence intervals) for the angiographic variables using the two restenosis criteria (≥0.72 mm loss in minimal luminal diameter from immediately post stenting to follow-up and diameter stenosis ≥50% at follow-up). The relative risk is indicated by the thick vertical line in the center and the outside vertical line represent the 95% confidence limits. The hatched vertical line signifies a relative risk of 1 (no additional risk for restenosis). Variables with values greater than or less than 1 imply additional or a reduction in risk respectively (see text for details). The variables are listed in the left hand column. N represents the number of lesions analyzed for each particular variable. Although 214 lesions were analyzed in total, some lesions could not be analyzed for certain variables. The denominator for vessel branch (111) represents the total number of lesions that were stented in native vessels. (CI, confidence interval; DS, diameter stenosis; LAD, left anterior descending artery; DIAM., diameter; PRIM., primary; PTCA, percutaneous transluminal coronary angioplasty; MLD, minimal luminal diameter; /, absolute change; MULT., multiple)*

DISCUSSION

Despite progress in techniques and equipment, the rate of late angiographic narrowing following PTCA, a process popularly termed "restenosis", has not been altered since its clinical introduction 13 years ago. This failure has provided the impetus for the development of newer alternative forms of coronary revascularization such as stenting, atherectomy and laser. However the effectiveness of all forms of nonoperative coronary interventions remains limited by the restenosis process(es).

Restenosis is a complex process that is only partially understood. Pathological studies of patients who have died more than 1 month following angioplasty have demonstrated the presence of intimal hyperplasia, presumably due to proliferation and migration of medial smooth muscle cells into the intima, and associated production of extracellular matrix collagen and proteoaminoglycans (13,14). It has been suggested by Liu et al that the two major factors that determine the absolute amount of intimal hyperplasia are (1) the depth of injury and (2) the regional flow characteristics (which are determined by the geometry of the dilated lumen of the lesion and blood flow velocity patterns across that lumen) (15). Two separate PTCA follow-up reports support the concept that the greater the diameter change post PTCA (implying a greater degree of disruption to the vessel wall), the more extensive is the absolute amount of reactive hyperplasia (16,17). On the basis of several angiographic studies from the Thoraxcenter, immediate results following stent implantation are superior to angioplasty alone (mean minimal luminal diameter of 2.5 mm versus 2.0-2.1 mm) and thus favor a more aggressive proliferative response post procedure (2,4,18). The second factor is illustrated by the inverse relationship between the level of wall shear stress and subsequent intimal thickening. In the presence of a significant residual stenosis, the post stenotic region is a site of flow separation and low wall shear stress. This may retard endothelial recovery and prolong the period of smooth muscle cell proliferation which is partially dependent on restoration of regenerated endothelial barrier (19). Stenting appears to diminish the effect of post stenotic wall shear stress by significantly improving the hemodynamic effects of the stenosis (based on the calculated reductions in Poisseuille and turbulent contributions to flow resistance) (20).

It is extremely difficult if not impossible to predict restenosis in the individual patient following PTCA (21). This problem can be partially understood when one considers that the two factors (ie. depth of injury and regional flow characteristics) affecting the extent of intimal proliferation act in opposition to the other and thus make it hazardous to predict outcome of this interaction in a particular patient. In large population of patients, relative risk analyses following PTCA have identified several patient, lesion, and procedural variables that predict late restenosis. However the situation following stenting may be different where the mean loss of minimal luminal diameter at late follow-up is twice that of PTCA alone (0.62 mm versus 0.31 mm) (2,18). Therefore, this study was designed to identify factors that were associated with an increased risk of restenosis following stenting.

Lesion Factors

Stented bypass grafts had a greater risk of restenosis than native vessels (30% versus 19%) but this finding was restricted to the DS criterion. The increased susceptibility of bypass grafts to the restenosis process has previously been documented following PTCA (9,22-26). Although left anterior descending (LAD) lesions have been shown to be a risk factor in several PTCA studies (5,15,16), this was not evident in our study. The reference diameter of the vessel also had no relationship to restenosis. Forty-three percent of the vessels had reference diameter between 3-4 mm and 43% were 3 mm or less. Lesion length and the severity of the lesion, in absolute minimal luminal diameter or diameter stenosis, prior to the procedure have been cited by several authors as important risk factors for restenosis following angioplasty although our data did not show this association (6-9). Lesion length is probably not an important factor for restenosis if lesions can be covered by a single stent (see below). We believe that this is due to a more uniform and optimal dilatation with stenting. Long lesions treated with angioplasty are frequently less successfully dilated along the entire length of the lesion and the ragged irregular surface of the vessel may predispose to rheological factors critically involved in restenosis. Total occlusions have been reported as an important predictor of restenosis in angioplasty studies. However, this accounted for only 4.5% of the lesions in our study which was too few for this analysis. Although there was a trend for higher restenosis in more eccentric lesions, this was not statistically significant.

Stent Factors

Multiple stents (RR: MLD 1.56 (1.08-2.25); DS 1.61 (0.97-2.67)) and unconstrained stent diameter exceeding reference diameter by > 0.7 mm (RR: MLD 1.64 (1.10-2.45); DS 1.93 (1.13-3.31)) significantly predicted restenosis with both criteria. Preliminary reports from four separate groups working with the Palmaz-Schatz stent have shown a similar relationship between multiple stents/lesion and restenosis (27-30). In our study, multiple stents placed in tandem were overlapped at the extremities (so called "telescoping") which may be the reason for the observed increase in restenosis rates. The segment of the vessel that was covered by the overlapping stents was subjected to the dilating force of two separate stents as well as an increased density of metal. We have observed that restenosis commonly occurred at these sites of overlapping between extremities of stents. Since the length of the lesion and the absolute length of the stent required to cover a lesion were not significant predictors, it seems prudent to implant longer stents rather than two or more shorter stents in tandem.

Selecting an oversized stent (unconstrained diameter > 0.7 mm larger than the reference diameter) was a particularly important stimulus for hyperplasia with the self-expanding Wallstent. Schwartz et al have described an aggressive proliferative response in a porcine model as a result of severe stent oversizing (0.5 to 1.5 mm) (31). This effect, which they attributed to penetration of the internal elastic lamina by the stent wires and subsequent deep medial injury, was much less pronounced when the stent diameter was matched more closely to the vessel diameter. Furthermore, due to its self expanding property, the Wallstent (and particularly when it is oversized) continues to expand the vessel wall for at least 24 hours post implantation (32). The vessel is subjected to increasingly higher wall stress than after

implantation of a balloon expandable stent (which is maximally expanded at the time of implantation), a factor which may adversely stimulate the proliferative process. It may seem paradoxical that oversizing the stent by >0.7 mm would result in a higher restenosis rate with the 50% DS criterion. However, the diameter stenosis post stent was not different in the two groups despite the oversizing. The main effect of oversizing then was not particularly a superior immediate result but rather a more aggressive "hyperplastic" reaction and a smaller MLD at follow-up than if less oversized stents were implanted. The absolute value of the unconstrained stent diameter and the addition of the polymer coating (Biogold®) had no significant relationship to late restenosis.

Procedural Factors

No significant relative risk could be attributed to a particular indication for the procedure. Restenosis rates for primary cases were not significantly different than for bail-out or restenosis cases (MLD Criterion:37%,42%,33%; DS Criterion: 24%,27%,24%) although an increased rate of restenosis has been described with the Palmaz-Schatz stent in patients with previous restenosis (30). The absolute change in diameter from the pre to the post stent result and dilatation within the stent after implantation (the so-called "Swiss Kiss") did not appear to affect the late restenosis. This post stent dilatation was performed to dissipate clot within the stent and to accelerate early expansion of the stent. A post stent diameter stenosis > 20% tended to be predictive of a follow-up diameter stenosis > 50% (RR 1.51, 95% CI 0.91-2.50) although not for the MLD criteria. The larger the residual stenosis following stenting (ie. less optimal result), the less hyperplasia is required to reach a reach a particular diameter stenosis at follow-up such as the 50% diameter stenosis criterion.

Limitations of Study

Several important limitations of this study must be mentioned. Although this study suggest several factors that may be predictive of restenosis following stenting, it does not address the actual mechanisms of restenosis in the stented vessel. By comparing the predictors of restenosis following stenting to angioplasty, we have assumed that the underlying mechanism(s) responsible for late angiographic narrowing are similar (i.e. primarily intimal hyperplasia). Although almost every stenting procedure was accompanied by balloon dilatation at some particular time during the procedure, several other mechanisms may be important. Elastic recoil, which in the first few days following the procedure may be a significant factor in causing renarrowing, may be less important in stented vessels than angioplasty alone due to the scaffolding function of the stent. Although organization of thrombus at the site of intimal damage following PTCA has been recognized as a cause for late restenosis, it has not been particularly regarded as an important factor based on late pathological studies following PTCA. However this may be an extremely important cause of late restenosis after stenting. Although it is difficult to histologically discriminate thrombus organization from intimal hyperplasia, we have observed a disorganized layer of intimal thickening directly above the stent wire associated with remnants of

thrombus in segments of several bypass grafts that have been surgically retrieved up to 10 months following stent implantation (33,34) (Figure 4). Therefore we consider organization of residual thrombus to be a potentially important cause of late angiographic narrowing in addition to the major occlusion problems early after stenting. This may partially explain why commonly regarded determinants of restenosis following PTCA (eg. lesion length, Left Anterior Descending Artery) do not appear to be significant in this analysis since a different pathological processes may predominate. This also has important clinical implications since therapy to limit smooth muscle proliferation may be quite different than therapy to minimize thrombus formation.

There are two statistical limitations to this study. Due to the relatively small sample size, we can not rule out a significant beta error. Secondly, in performing multiple statistical comparisons, there is a risk that some of them may be significant by chance alone. Therefore, this data requires confirmation by other studies.

In conclusion, the European coronary Wallstent experience has demonstrated that restenosis following stenting is increased in bypass grafts and in the presence of multiple stents and excessive oversizing of the stent (>0.7 mm) and less optimal results immediately post stenting (>20% diameter stenosis). Since some of these factors can be modified, we recommend against the use of multiple stents and excessive oversizing to reduce the probability of late restenosis.

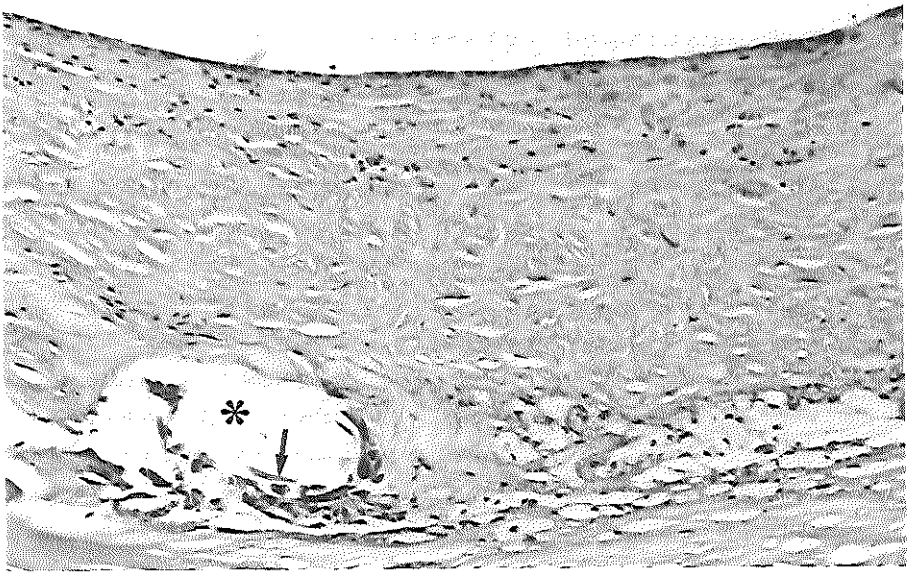

Figure 4. *Light micrograph of stented bypass graft removed 10 months after stent implantation. The void (*) represents a 70 micron diameter stent wire. Immediately adjacent to the stent wire are cellular debris and foam cells (arrowhead). Directly above the stent wire is a layer of disorganized fibrointimal hyperplasia. (courtesy of HMM van Beusekom)*

Acknowledgement

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Table 1. Discrete parameters

Lesion related	Vessel Type (Native / Bypass)
	Vessel Branch (LAD / non LAD)
Stent related	Stent Type (Polymer Coated / Noncoated)
	Stent Number (Single / Multiple)
Procedural related	Indication (Primary / Restenosis / Bailout)
	Intrastent Dilatation ("Swiss Kiss" / No SK)

LAD = left anterior descending coronary artery

Table 2. Continuous parameters

		Median
Lesion related	Obstruction diameter (mm) -Pre	1.20
	Diameter stenosis (%) -Pre	60
	Lesion length (mm)	8.0
	Reference diameter (mm)	3.25
	Symmetry	0.85
Stent related	Unconstrained stent diameter (mm)	4.0
	Unconstrained stent length (mm)	20.0
	Oversize (mm)	0.7
Procedure related	Change in obstruction diameter (mm)	1.3
	Diameter stenosis (%) -Post Stent	20

Table 3. Restenosis rates according to Criterion 1. (≥ 0.72 mm Loss in Minimal Lumen Diameter)

Parameter		n	Restenosis Rate
Stent Number	Multiple	22/44	50%
	Single	53/165	32%
Stent Oversize	> 0.7	40/90	44%
	≤ 0.7	26/96	27%

Table 4. Restenosis Rates according to Criterion 2. (> 50% Diameter Stenosis at Follow Up)

Parameter		n	Restenosis Rate
Vessel Type	Bypass	30/103	30%
	Native	20/111	19%
Stent Oversize	> 0.7	29/90	32%
	≤ 0.7	16/96	17%
Diameter stenosis- Post Stent	> 20%	30/107	28%
	≤ 20%	19/102	19%

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Chapter 7

Quantitative Angiographic Follow-up of the Coronary Wallstent[R] in Native Vessels and Bypass Grafts (European Experience March 1986-March 1990)

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Am J Cardiol (accepted)

Abstract

Coronary stenting has been investigated as an adjunct to percutaneous transluminal coronary angioplasty (PTCA) to obviate the problems of early occlusion and late restenosis. From March 1986-March 1990. 265 patients (308 lesions) were implanted with the coronary Wallstent^{R} in six European centers. For this study, the patients were analyzed according to date of implantation (Group 1:March 1986-January 1988; Group 2: February 1988-March 1990) and vessel type (native arteries versus bypass grafts). Quantitative angiographic follow-up was performed in 82% of the study patients.

The early in-hospital occlusion rate in the overall group was 15%. Group 1 patients had a 20% rate in contrast to 12% rate in Group 2 (p=NS). The early occlusion rate in native vessels and bypass grafts was 19% and 8% respectively (p=0.019). Restenosis was determined by two criteria (Criterion 1: ≥0.72 mm loss in minimal luminal diameter (MLD) from post stent to follow-up; Criterion 2: ≥50% diameter stenosis at follow-up) within the stent and in the segments immediately proximal and distal to the stent. The restenosis rate with Criterion 1 was 43% in the overall group of patients; 35% in Group 1 versus 49% in Group 2 (p=NS); 34% in native vessels versus 54% in bypass grafts (p=0.016). The second criterion was met by 27% of patients in the overall group; 21% in Group 1 versus 32% in Group 2 (p=NS); 18% in native vessel patients versus 39% in bypass grafts (p=0.005).

The overall mortality during the study period was 6.6% in native vessel patients and 8.9% in patients with bypass grafts (6% and 7.9% at 1 year respectively). The actuarial event-free survival (freedom from death, myocardial infarction, bypass surgery or PTCA) for native artery patients was 46% at 40 months and for bypass graft patients was 37% at 20 months.

It is concluded that early in-hospital occlusions remain a major problem with this device despite improvement in the later experience. Although bypass grafts had a significantly lower early occlusion rate than native vessels, a significantly higher rate of late restenosis limited the early benefits of stenting. Restenosis occurs in a significant number of patients, particularly in bypass grafts. The indications for stenting remain unknown and require results of randomized clinical studies.

Key Words:stents, restenosis, coronary artery disease

INTRODUCTION

In 1986, the first coronary Wallstent implantation ushered in a new era in interventional cardiology with the purpose of circumventing the two major limitations of coronary angioplasty, early acute occlusion and late restenosis (1). As with all new procedures, operators of the device had to struggle with their own learning curves at the same time that anticoagulation regimens and clinical indications and contraindications evolved from their clinical experience. In May 1988, the five European centers testing this device agreed to set up a core laboratory for quantitative angiographic analysis in Rotterdam to objectively assess the results. In a previous publication from our group, the late angiographic and clinical follow-up of the inital 105 patients (2). These stent implantations were predominantly performed in native coronary vessels. In the period from February 1988 until March 1990, a further 160 patients underwent stent implantation in the coronary circulation. This second group was characterized by a predominance of bypass vessels and a different set of indications. In this report, we compare the late quantitative angiographic and clinical follow-up of this second group of patients with the initial group, and for bypass grafts versus native vessels. In particular, we were interested to determine whether the changing patterns of patient selection and management based on the initial experience, resulted in improved rates of early occlusion and late restenosis and whether the results of stents implanted in bypass grafts differed from native vessels.

METHODS

Study Patients

Two hundred and sixty-five patients (308 lesions) were enrolled after obtaining informed consent between March 1986 and March 1990 at the participating centers. The study protocol was approved by the individual hospital ethics committees. The mean age of the population was 58 ± 11 years and 84% were male. The study patients were grouped according to the date of implantation (March 1986-January 1988, February 1988-March 1990 Groups 1 and 2 respectively) and the vessel type stented (native vessel versus bypass graft) (Tables 1,2). The first period of stent implantations, until January 1988, has previously been reported (2). Almost 90% of the first group were implanted at either Lausanne (n=56) or Toulouse (n=32). However, in the second period, about 80% of the patients were implanted in Rotterdam (n=80) and in Lausanne (n=43). In Group 1, 117 stents were implanted in 114 lesions, of which 82% were in native vessels (and in particular the left anterior descending artery). In group 2, 266 stents were implanted in 194 lesions, predominantly in bypass grafts (60% of cases).In this group, the right coronary artery was the most common vessel stented in the native circulation. Two patients had stents placed in both a bypass graft and a native vessel. The indications for stenting also differed between the two vessel types (Figure 1).

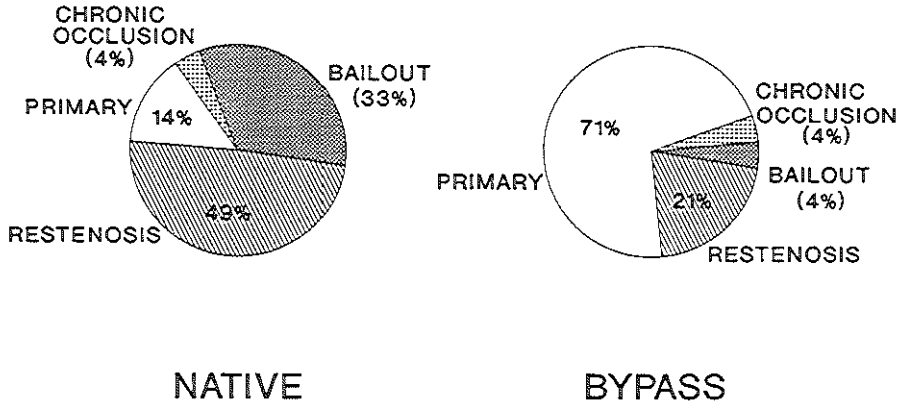

Figure 1. *The indications for stenting in native arteries and bypass grafts. (Primary = Primary atherosclerotic lesion that has not been previously treated by PTCA or stenting.)*

Native vessels were primarily stented to prevent a second restenosis or as a bail-out procedure for angioplasties complicated by abrupt closure or large dissections that interrupted anterograde flow and were associated with clinical and electrocardiographic signs of ischemia. However in bypass grafts, the principle indication was for primary lesions in bypass grafts that had not been previously treated with angioplasty.

In this trial, the endovascular prosthesis, Wallstent^{R}, was provided by Schneider Europe, Zurich. The method of implantation and description of this stent has previously been reported (1,2,5-7). Unconstrained stent diameter ranged from 2.5-6 mm and was selected to be 0.50 mm. larger than the stented vessel. In an effort to alleviate the problem of acute thrombosis, the stent design was changed in April 1989 with the introduction of a polymer coated stent (Biogold^{R}) for some stent sizes. By August 1989, all manufactured stents contained this particular polymer coating.

The anticoagulation for the first period of implantation has previously been described (2). Based on this initial clinical experience, a uniform anticoagulation schedule was followed at the centers. Acetylsalicylic acid 1 gram orally was started 1 day before the procedure. At the beginning of the procedure, patients recieved heparin 10,000 international units intravenously and in some cases, dextran infusions (500 mg/ 4 hours) were also given. Additional heparin (10,000 international units) and urokinase 100,000 units intracoronary were administered during the procedure. Following the procedure,

the heparin infusion was adjusted according tho the activated partial thromboplastin time (APTT 80-120 seconds) in addition to initiating oral Vitamin K antagonist therapy. Heparin was discontinued after the therapeutic oral anticoagulation level was stabilized (International Normalized Ratio of 2.3 or more). Acetylsalicylic acid 100 mg daily, dipyridamole (300 to 450 mg/day) and in some patients sulfinpyrazone 400 mg daily were also administered.

Quantitative Coronary Arteriography and Restenosis Criteria

All cineangiograms were analyzed at the core laboratory in Rotterdam using the computer assisted cardiovascular angiography analysis system (CAAS) which has previously been discussed in detail (3,4). The minimal luminal diameter and % diameter stenosis were determined pre and post angioplasty, immediately post stent implantation and at long term follow-up in all patients using the average of multiple matched views with orthogonal projections wherever possible. The minimal luminal diameter (and the diameter stenosis using the reference diameter of the segment) of each segment immediately proximal and distal to the stent was also measured.

Two different set of criteria were applied to determine the restenosis rate. We have found a change in minimal luminal diameter (MLD) of 0.72 mm or more to be a reliable indicator of angiographic progression of vessel narrowing and by no means implies functional or clinical significance(3,4,8). This value takes into account the limitations of coronary angiographic measurements and represents two times the long term variability (ie. the 95% confidence intervals) for repeat measurements of a coronary obstruction using CAAS. The other criterion for restenosis chosen was an increase of the diameter stenosis from less than 50% after stent implantation to greater than or equal to 50% at follow-up. This criterion was selected since common clinical practice continues to assess lesion severity by a percentage stenosis. The two criteria were assessed within the stent and in the segment immediately adjacent (proximal and distal) to the stent.

In this study, late (ie. documented after the initial discharge from hospital) occlusion (n= 10 patients, 16 lesions) were regarded as restenoses. This contrasts with our earlier report in which we calculated a total occlusion rate which was then separated into early (in hospital) and late occlusions (2). In this earlier report, the early and late occlusions were analyzed separately from the restenotic lesions. The current change was initiated to be consistent with our reports of late follow-up of PTCA (4,9).

Statistical Methods

The data obtained by quantitative angiographic analysis are given as mean ± standard error of the mean. The means for each angiographic variable pre PTCA, post stent and at follow-up were compared by analysis of variance. If significance differences were found, two tailed T-tests were applied to pairs of data. The occlusion and restenosis rates were compared using a chi square test. A statistical probability of less than 0.05 was considered significant.

The late clinical follow-up was determined according to a life table format using the Kaplan-Meier

method (10). The following events were considered clinical endpoints: death, myocardial infarction, bypass surgery or nonsurgical revascularization (PTCA or atherectomy). The life table was constructed according to the initial clinical event.

RESULTS

The angiographic follow-up for the entire study population was 82% (Table 3). This includes patients with documented early occlusions during hospital admission (n=40) in addition to patients who had late (after the initial hospital discharge) angiographic controls. The reasons why follow-up angiography could not be performed are listed in Table 3. The time to angiographic follow-up was 6.6 ± 4.8 months if early occlusions are excluded and 5.7 ± 5.0 months with the early occlusions.

The angiographic data for individual lesions in bypass grafts and native vessels is presented in Figures 2 and 3. In native vessels, there was a mean increase in minimal luminal diameter from 1.17 ± .04 mm to 2.53 ± .04 mm immediately post stenting ($p<0.0001$) but a late deterioration to 1.99 ± .07 mm ($p<0.0001$ versus the post stent result) if early occlusions are excluded and 1.59 ± .09 mm with the inclusion of the early occlusions ($p<0.0001$) (Figure 2a). Similarly, the minimal luminal diameter increased significantly in bypass lesions from 1.39 ± .06 mm to 2.81 ± .06 mm post stenting with a late reduction to 2.21 ± .11 mm and 2.03 ± .12 mm with the exclusion and inclusion of early occlusions, respectively ($p<0.0001$). Diameter stenosis was significantly reduced from immediately post stenting in bypass grafts from 60 ± 1% to 23 ± 1% but increased at late follow-up to 43 ± 3% and to 38 ± 3% with and without the early occlusions respectively ($p<0.0001$ versus the post stent result). Similar changes were observed in native vessels (Figure 2b).

In the overall group, the incidence of early in-hospital occlusion was 15% by patient and 13% by lesion (Table 4). In bypass grafts, early occlusions were documented in 7% of lesions (8% of patients) versus 18% of lesions (19% of patients) in native vessels (by lesion $p=0.005$;by patient $p=0.016$). Three of these native vessel occlusions occurred during the procedure and could not be recanalized. The remaining occlusions presented clinically as acute ischemic syndromes following a successful stenting procedure. Early occlusions were less frequent in Group 2 patients (12%) than in Group 1 (20%) but not statistically significant. Detectable angiographic narrowing (0.72 mm loss in MLD) in the overall group was 42% by lesion and 43% by patient (Table 5). Using the 50% diameter stenosis criterion, restenosis occurred in 27% of lesions (27% of patients). Restenosis according to either definition was significantly higher in bypass grafts (MLD criterion:54% by patient; DS criterion:39% by patient) than in native vessels (34% and 18% respectively) (MLD: $p=0.016$; DS: $p=0.001$). Group 2 patients (MLD criterion:49% by patient; DS criterion: 32% by patient) did not have significantly greater restenosis than Group 1 patients (MLD criterion:35%; DS criterion:21%).

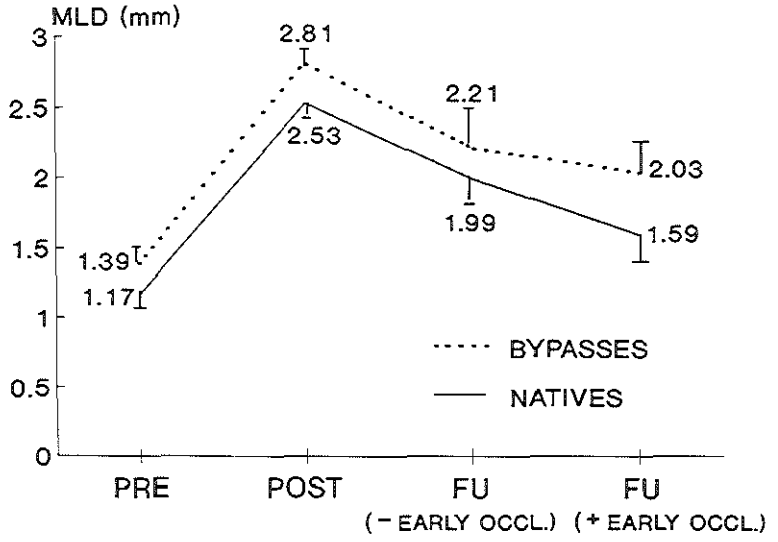

Figure 2a. *Minimal luminal diameter (MLD) of native vessels and bypass grafts pre procedure, post stenting, and at follow-up. The mean values at follow-up have been calculated with and without the inclusion of the early in-hospital occlusions.*

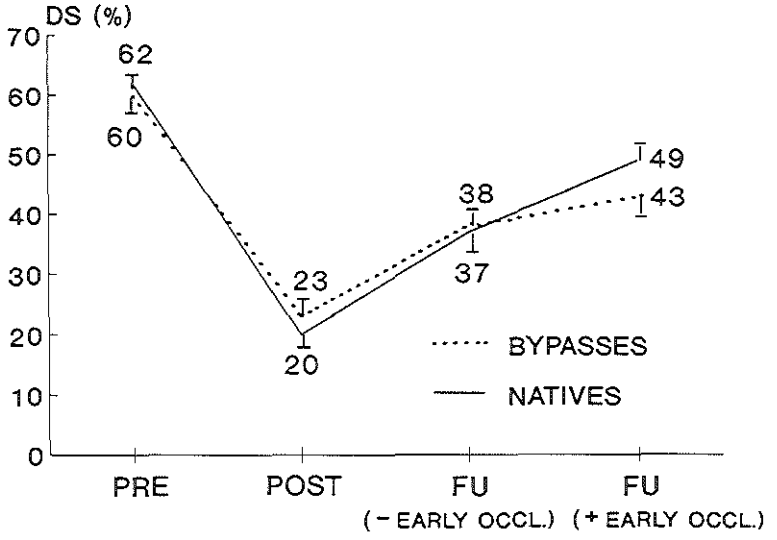

Figure 2b. *The % Diameter stenosis (DS) of native vessels and bypass grafts pre procedure, post stenting, and at follow-up. The mean values at follow-up have been calculated with and without the inclusion of the early in-hospital occlusions.*

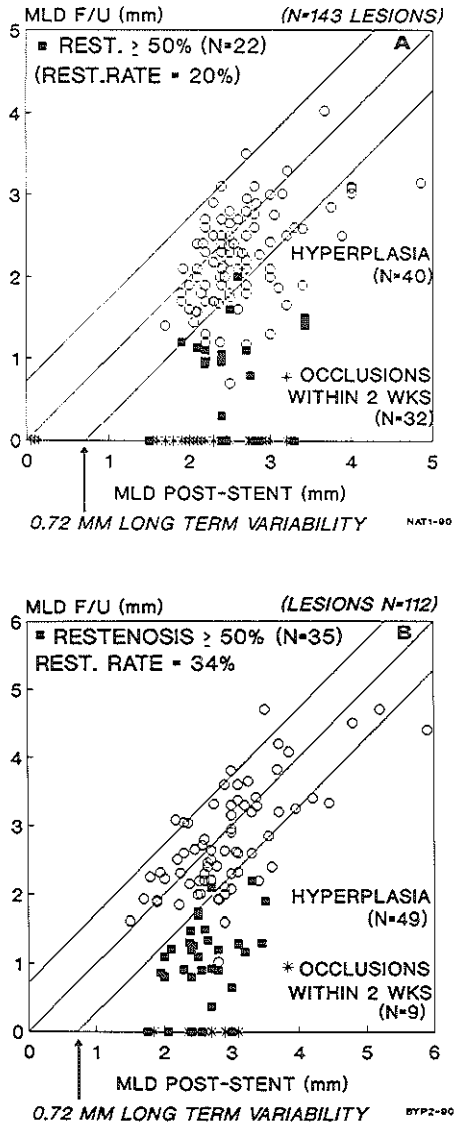

Figure 3. *Change in the minimal luminal diameter (MLD) for individual lesions in native vessels (Figure 3A) and in bypass grafts (Figure 3B) between stent implantation and angiographic follow-up (F/U). The diameter of each segment immediately after implantation is plotted against the diameter at follow-up. The lines on each side of the identity line (diagonal) represent the limits of long-term variability of repeat measurements (a change of ≥0.72 mm [arrow]). All symbols below the right-hand line represent stents with involvement of angiographic detectable hyperplasia. The filled squares represents lesions with follow-up diameter stenosis ≥ 50%. Occlusions are located along the x axis and those lesions that occurred within the first two weeks are marked by an asterisk.*

The overall mortality during the study period was 8.9% (n=9) for bypass grafts and 6.6% (n=11) for native vessels (7.9% and 6% at 1 year respectively). In the bypass group, four of the nine deaths occurred during the initial hospitalization. Two of these deaths resulted from intracerebral hematomas related to the anticoagulation and two were from myocardial infarctions due to stent occlusion. Two of the 5 late deaths were sudden (at 2 and 18 months), two were clearly unrelated to the stent (chronic congestive heart failure, chronic renal failure) and the other death occurred after bypass surgery. In the group with native vessels, 7 of the 11 deaths were in-hospital. These were all due to myocardial infarctions resulting from stent occlusion with the exception of one intracerebral bleed and one patient who was stented 24 hours after an extensive myocardial infarction with cardiogenic shock. Two of the four late deaths were sudden (at 1.5 and 19 months), one was noncardiac (pneumonia) and the other resulted from complications post bypass surgery. The actuarial event-free survival (freedom from death, myocardial infarction, bypass surgery or PTCA) for native artery patients was 46% at 40 months and for bypass graft patients was 37% at 20 months (Figure 4).

DISCUSSION

The coronary Wallstent was initially introduced as an endovascular device to prevent the late restenosis process that limits percutaneous transluminal coronary angioplasty. The indications for and management of patients implanted with this particular prosthesis have evolved as experience and knowledge have increased. In this study, an attempt has been made to separate two important factors in the late outcome of patients with stent implantations. The first division, according to date of implantation, provides the clearest picture of the changes in stent applications based on the early experience. Investigators originally believed that the stent could be safely implanted in native vessels and that the benefit of stents would be most apparent in lesions that had already restenosed on at least one occasion. However, a high in-hospital occlusion rate was noted, particularly in patients with unstable syndromes, evolving myocardial infarction or angiographic evidence of thrombus. These occlusions, often with disastrous clinical sequelae, convinced most of the investigators that native vessels in general and particularly in the left anterior descending artery (due to the large territory at risk), should only be stented in bail-out situations. Group 2 mainly consisted of patients with bypass grafts who were stented for primary lesions and native vessels who were stented as part of a bail-out strategy following complicated balloon angioplasty. Bypass grafts in particular were selected for stent implantations due to an extremely high rate of restenosis after PTCA alone and the larger diameter of these grafts seemed less likely to thrombose than smaller calibre native arterial vessels (11-14). Bypass lesions, which were the majority of lesions stented in Group 2, were more complex in general than Group 1 lesions due to the advanced age and diffuse nature of the disease in the bypass grafts. As a result, more stents per lesion (1.4 versus 1.1 in native vessels) were required to cover these lesions.

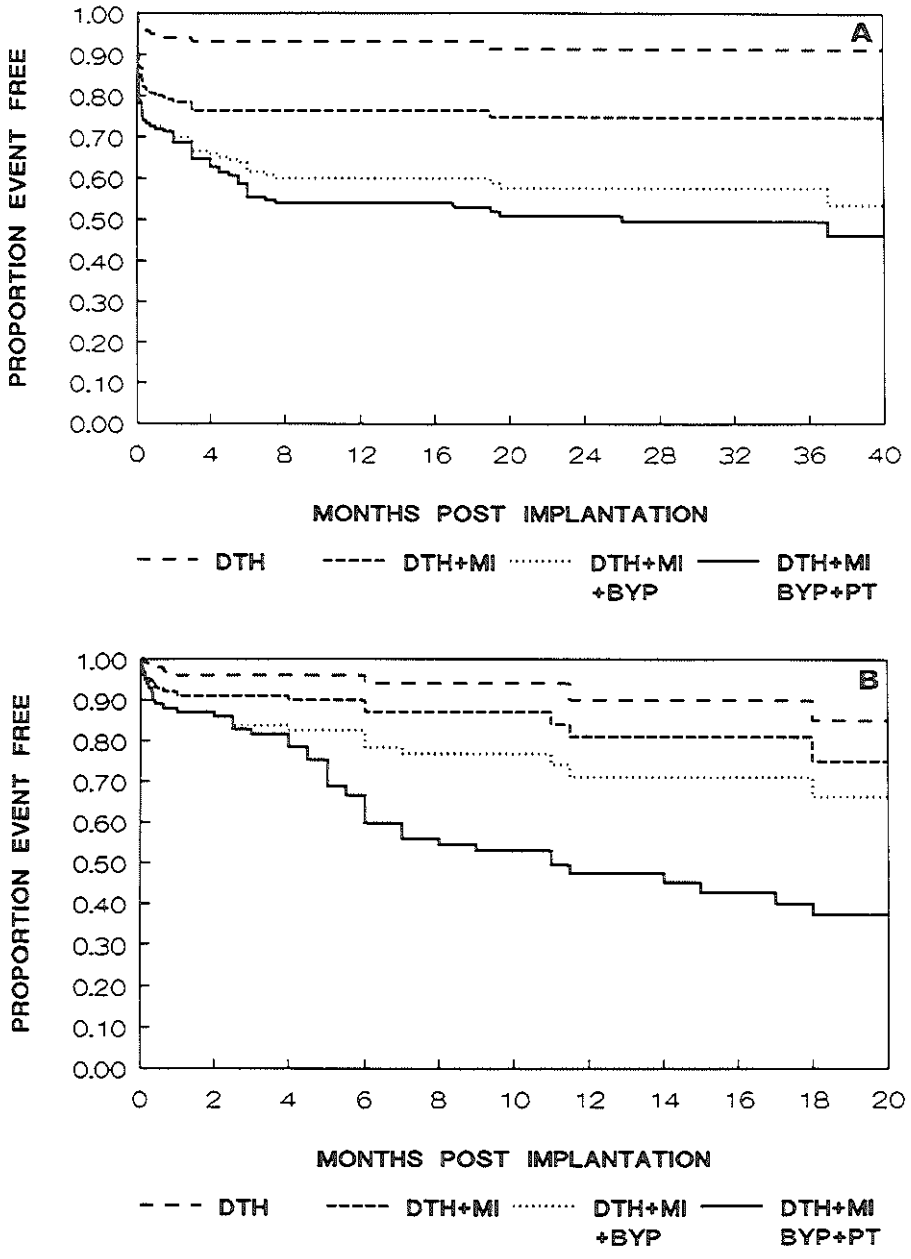

Figure 4. *Clinical follow-up in native vessels up to 40 months (Figure 4A) and bypass grafts up to 20 months (Figure 4B). Death, myocardial infarction, bypass surgery, and PTCA or atherectomy were considered clinical end points.*

Therefore, the significantly lower rate of in-hospital occlusion in bypass graft patients versus patients with native vessels (8% versus 19%) and trend in Group 2 versus Group 1 (12% versus 20%) is indicative of several possible factors including improvements in anticoagulation regimens, operator experience and/or larger calibre vessels despite more complex case selection.

Recently, the initial clinical experience with the Palmaz-Schatz stent has been reported (15). Using a similar anticoagulation schedule in 174 patients, a 0.6% in hospital occlusion rate was demonstrated. The discrepancy between a substantially higher occlusion rate in our series with the Wallstent and the Schatz study can not be entirely explained. The stent itself does not appear to be more thrombogenic. In a model of stents placed inside a polytetrafluoethylene graft in exteriorized arteriovenous shunts in baboons, no difference in acute platelet deposition and thrombus formation was noted between the two types of stents (16). Differences in study design such as patient selection (collateralized vessels, predominantly right coronary arteries and exclusion of patients with recent myocardial infarction and abrupt closure following PTCA in the Schatz study) may account for some of the differences.

Higher restenosis rates by both criteria were demonstrated in bypass grafts compared with native vessels and in Group 2 than in Group 1. There are two possible explanations for this increase. First, bypass grafts, which are overrepresented in Group 2, are known to have higher restenosis rates than native vessels (11,12,13). Secondly, higher restenosis rates may be the "price" for lower occlusion rates. Organization of thrombus at the site of intimal damage may be an important cause of late restenosis after stenting. Although it is often difficult to histologically differentiate thrombus organization from intimal hyperplasia, we have observed an extremely disorganized pattern of intimal thickening in the stented segments of several bypass grafts that have been surgically retrieved or obtained by atherectomy 1-5 months following stent implantation (17). By diminishing the formation of early occlusive thrombus with more effective anticoagulation, the residual non occluding thrombus could form the substrate for late restenosis. Although the second group had a higher proportion of bail-out cases, a previous study from our group did not identify increased relative risk for restenosis from bail-out cases in comparison to stent implantations performed in primary or restenosed lesions (18).

The problems of prolonged anticoagulation are an additional consideration. Increased morbidity (increased femoral hematomas, gastrointestinal and genito-urinary tract bleeding) and mortality (3 patients died from intracerebral hemorrhage) are directly attributable to the intensive anticoagulation regimen. The duration of hospitalization is also lengthened to ensure therapeutic levels of anticoagulation.

The high incidence of late adverse clinical events in stented patients is a cause for concern. A mortality rate of 8.9% in bypass grafts and 6.6% in native vessels is higher than in reported PTCA studies (19,20,21). However, it must be stressed that a large number of stents in native vessels were implanted for abrupt closure following PTCA which dramatically increases the risk of the procedure (21). Actuarial event free survival (freedom from death, myocardial infarction, bypass surgery or repeat PTCA or atherectomy) was 37% at 20 months in bypass patients and 46% at 40 months in native vessels. In the

bypass group, about 30% of the adverse events were unrelated to the stented lesion and were due to worsening of a different lesion or to development of new lesions. In the native vessel group, 12% of the adverse events were unrelated to the stented lesion. In addition, 9 of the 30 bypass operations in stented native vessel patients were performed as part of a protocol for patients stented for the bail-out indication (22). Although there are no comparable series of native vessel patients in the literature because of the unique set of indications in our study, three recent reports have been published of late clinical follow-up (Kaplan-Meier analysis) after PTCA in bypass grafts. The Thoraxcenter reported that only 41% of patients were alive and event-free (myocardial infarction, repeat CABG, repeat PTCA) at a median follow-up of 2.1 years (23). A review of the overall Dutch experience also showed limited late beneficial results with a two year and five year event free survival of 52% and 26% respectively in 454 bypass patients (24). Webb et al have described a 71% freedom from death, infarction and surgery at 5 years in bypass patients who underwent PTCA at their institution but did not include the 27% incidence of second angioplasty procedures also required in their patient group (11). However it must be stressed that our study was not a randomized trial designed to compare stenting with PTCA but rather an observational study with a first generation coronary stent. Nevertheless, all of these late follow-up studies of nonoperative coronary revascularization clearly show that these are palliative procedures and not long-term solutions to the underlying problems of progression of underlying coronary disease and iatrogenically induced restenosis.

Several important points emerge from this study. First, although in hospital occlusion rates improved in the later experience, Wallstent^{R} coronary thrombosis continues to limit its use. Restenosis rates with the 50% DS criterion do not seem to be significantly improved when compared with historical post angioplasty results, although definitive statements must await randomized trials. Bypass grafts in particular have a high incidence of late restenosis rate although early occlusion occurred less significantly than in patients with native vessels. Based on our experience, there is insufficient evidence at this time to suggest implantation of this particular stent outside of a randomized trial with the following exceptions: (1) bail-out for abrupt occlusion, (2) suboptimal (inadequate dilalatation) results following PTCA, and (3) bypass grafts at high risk for distal embolization with PTCA (friable lesions that may benefit from the scaffolding property of the Wallstent^{R}). If the low occlusion rate with the Palmaz-Schatz stent is confirmed in other studies, that particular stent would appear to be a more suitable candidate for randomized trials of presently available stents.

Table 1: Stent implantations according to date of implantation.

	Group 1 (March 1986-Dec 1988)	Group 2 (Jan 1989-March 1990)
Vessels	107	175
Bypass	18%	60%
Native	82%	40%
LAD	54%	15%
CX	7%	5%
RCA	21%	20%
Stent/Lesions	117/114	266/194

Table 2: Stent implantations according to vessel type.

	Natives	Bypass Grafts
Patients	166	101
Vessels	171	110
Stent/Lesion	193/173	192/135
LAD	55%	
CX	10%	
RCA	35%	

Table 3: Angiographic Follow-up

Implantations (Lesions/Pts)	308/265	
Early Occlusions	41 / 40	
Late Follow Up	214/176	
Total	255/216	(82%)
No Angiographic Follow-up	53 / 49	(18%)
Death	10	(4%)
Early CABG	11	(4%)
Refusal	25	(9%)
Technical	3	(1%)
Time to Angiographic Follow Up		
Excluding Early Occlusions	6.6 ± 4.8	
Including Early Occlusions	5.7 ± 5.0	

Table 4: Angiographic Results: Early Occlusions

	Total	Group 1	Group 2	Native Vessels	Bypass Grafts
Lesions	308/265	114/105	194/160	173/166	135/101
Early Occlusions	40 (13%) / 39 (15%)	21 (18%) / 21 (20%)	20 (10%) / 19 (12%)	32 (18%) / 32 (19%)	9 (7%) / 8 (8%)

Table 5: Late Angiographic Follow Up: Restenosis within and immediatly adjacent to the Stent.

	Total	Group 1	Group 2	Native Vessels	Bypass Grafts
Lesions/Patients	214 / 176	85 / 75	129 / 101	111 / 104	103 / 74
0.72mm Criterion					
Within Stent	75 / 61	25 / 21	50 / 40	34 / 30	40 / 31
Adjacent to Stent	14 / 14	5 / 5	9 / 9	5 / 5	9 / 9
Total	89 / 75	30 / 26	59 / 49	40 / 35	49 / 40
	(42% / 43%)	(37% / 35%)	(46% / 49%)	(36% / 34%)	(48% / 54%)
50% DS Criterion					
Within Stent	51 / 42	17 / 15	34 / 27	21 / 18	30 / 24
Adjacent to Stent	6 / 6	1 / 1	5 / 5	1 / 1	5 / 5
Total	57 / 48	18 / 16	39 / 32	22 / 19	35 / 29
	(27% / 27%)	(22% / 21%)	(30% / 32%)	(20% / 18%)	(34% / 39%)

Appendix

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Chapter 8

Stenting of venous bypass grafts: A new treatment modality for patients who are poor candidates for reintervention

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(submitted)

Abstract

During a two year period, 136 self-expanding Wallstents (Medinvent, Lausanne) were implanted in saphenous vein bypass grafts in 69 patients with end stage coronary artery disease. All patients had severe symptoms and the majority were poor candidates either for repeat surgery or conventional bypass coronary angioplasty due to unfavorable native anatomy, impaired left ventricular function or a high risk bypass lesion anatomy for coronary angioplasty.

All procedures were technically successful without major complications and need for emergency bypass surgery. However, during hospital stay, acute thrombotic complications occurred in 7 patients (10%) resulting in one death and acute myocardial infarction in 5 patients and necessitating emergency rePTCA in 2 patients and reCABG in 4 patients. Twenty three patients had serious hemorrhagic complications directly related to the rigorous anticoagulation schedule. Two patients died due to fatal cerebral bleeding.

During follow-up, another five patients died accounting for a total mortality rate of 12%. At late angiographic follow-up (4.9 ± 3.4 months, n = 53), 25 patients (47%) developed a restenosis (≥ 50% DS) within or immediately adjacent to the stent, necessitating reintervention in 19 patients (PTCA: n = 12; reCABG: n = 7). In the patient group without stent related restenosis (n = 28), 15 patients developed progression of disease in either the native or bypass vessels leading to recurrence of major anginal symptoms, within 1 to 24 months. Ten of these patients required further intervention (Stent: n = 6; PTCA: n = 3; reCABG: n = 1).

Stenting in saphenous coronary bypass grafts can be performed safely with excellent immediate angiographic and clinical results. Early occlusion, late restenosis and bleeding complications associated with the aggressive anticoagulant treatment remain significant limitations. Reintervention due to restenosis or progression of disease in other lesions is common. Stenting of diseased bypass grafts in symptomatic patients with end stage coronary artery disease (who are at high risk for conventional angioplasty or surgical reintervention) may be useful as palliative therapy.

INTRODUCTION

Patients presenting with medically refractory anginal symptoms following saphenous vein bypass surgery pose a difficult problem for cardiologists and cardiovascular surgeons. These patients are generally older, with more extensive, diffuse disease involving the native coronary arteries and venous bypass grafts. Repeat coronary bypass surgery for recurrent ischaemia is technically more difficult, is associated with a higher mortality and morbidity, and has inferior long-term clinical results when compared to a first bypass operation (1-3). Conventional balloon angioplasty offers an alternative mode of revascularization in selected patients. The immediate results of this procedure have been shown to be favourable in patients with discrete lesions in venous bypass grafts, but considerably less satisfactory in diffusely diseased, ulcerated, or thrombosed venous grafts (4-6). Furthermore, it appears that the restenosis rate is high, varying from 40-70% depending on the site of the lesions in the graft and the overall extent of disease in the conduit (7-13).

Stent implantation has been proposed as an alternative or adjunct to PTCA for diseased venous bypass grafts. Early studies with small numbers of patients have shown that stents can be placed safely and successfully in bypass grafts with an encouraging low rate of restenosis (14-21). Therefore, we initiated this observational study to assess the acute and late results of stent implantation in stenosed coronary artery bypass grafts in symptomatic patients with diffuse extensive native coronary artery and bypass graft disease who represent poor candidates for conventional balloon angioplasty or re-operation.

METHODS

Study population (Tables I and II)

Between January 1988 and March 1990, 136 stents were implanted in 69 (12 female, 57 male) patients in the four participating hospitals in the Netherlands and Belgium. The study protocol was approved by the individual hospital ethics committees and informed patient consent was obtained. A senior investigator (PWS) was present for all stent implantations.

The decision to implant a stent was reached after combined discussion between cardiologists and surgeons (Table III). Forty-two patients were considered inoperable, either due to high risk/benefit ratio related to repeat surgery (n=28), unfavourable coronary vessel anatomy such as diffusely diseased distal vessels (n=4), poor left ventricular function (ejection fraction < 35%) (n=6), or concurrent noncardiac risk factors (n=4). Conventional coronary angioplasty was considered high risk in 39 patients due to the age of the grafts, the length of stenosis and/or unfavorable angiographic features (tandem lesions, eccentric lesions, lesions containing ulcers, aneurysm, calcifications or dissections) (Figures 1-4).

The mean patient age was 63 years (range 44-78), and the age of the implanted bypass graft was 83 months (range: 1 - 166). Forty-eight patients had at least one previous myocardial infarction, and 8

had undergone more than one previous bypass procedure. Single vessel, double vessel and triple vessel disease were present in 3, 23 and 74% of the patients respectively. Eleven patients were in NYHA class II, 27 in NYHA class III, 29 in NYHA class IV and in 2 patients the stents were implanted during evolving myocardial infarction. All patients had documented electrocardiographic evidence of ischemia. The stent implantation data is presented in Table IV. A single stent was implanted in 30 patients, 2 stents in 24 patients, 3 stents in 7 patients, 4 stents in 5 patients, 1 patient received 5 stents and finally 2 patients had 6 stents implanted. The stent was placed in single grafts in 25 cases and in sequential grafts in 44 cases. In 5 patients, the stent was implanted into totally occluded vessels (during an evolving myocardial infarction (n=2) and in three chronic occlusions).

Implanted device

In the first 26 patients we used the Medinvent Wallstent^{R} (Medinvent, Lausanne, Switzerland), later we used the polymer coated Medinvent Biogold^{R} stent. The stent consists of a stainless steel alloy with a self-expanding mesh design (15). The unconstrained length varied between 15 and 27 mm and its diameter in fully expanded state was between 3.5 and 6.0 mm and was selected to be 0.50 mm larger than the reference diameter of the vessel.

After stent implantation, the patients were monitored in the coronary care unit. The rigorous anticoagulation regimen has previously been described (22). All patients received aspirin the day before the procedure and intravenous heparin (10,000 I.U) at the beginning of the procedure. Prior to stent implantation 10,000 I.U. heparin and dextran 500 mg/4 hrs, were given intravenously. Immediately after stent implantation, 100,000 - 250,000 I.U. urokinase was infused into the coronary bypass graft via the guiding catheter. Intravenous heparin administration was continued at a minimum dosage of 24,000 I.U./ 24 hrs; the dosage was adjusted according to the activated partial thromboplastin time (APTT: 80-120 sec). Oral acenocoumarol was started the day of implantation. The heparin infusion was continued until the prothrombin time measured by ThrombotestR (Nycomed, Oslo, Norway) (TT) was lowered to 5 - 10% for 2 subsequent days, and discontinued slowly thereafter. Following stent implantation, the patients were also given aspirin (300 mg/day), dipyridamole (300 - 450 mg/day), and nifedipine (30 - 60 mg/day) which in addition to the oral anticoagulant were maintained for 3 - 6 months after the procedure. The patients were followed at our out patient clinic at one and three months and underwent a repeat coronary angiogram 6 months after the initial procedure, or earlier if symptoms recurred.

Quantitative coronary angiography

The quantitative analysis of the stenotic coronary segments was carried out with the computer assisted Cardiovascular Angiography Analysis System (CAAS), which has been described in detail (23-28). The angiographic analysis was done pre- and postangioplasty, immediately post stent implantation and at follow-up in all patients using the average of multiple matched views with orthogonal projections wherever possible.

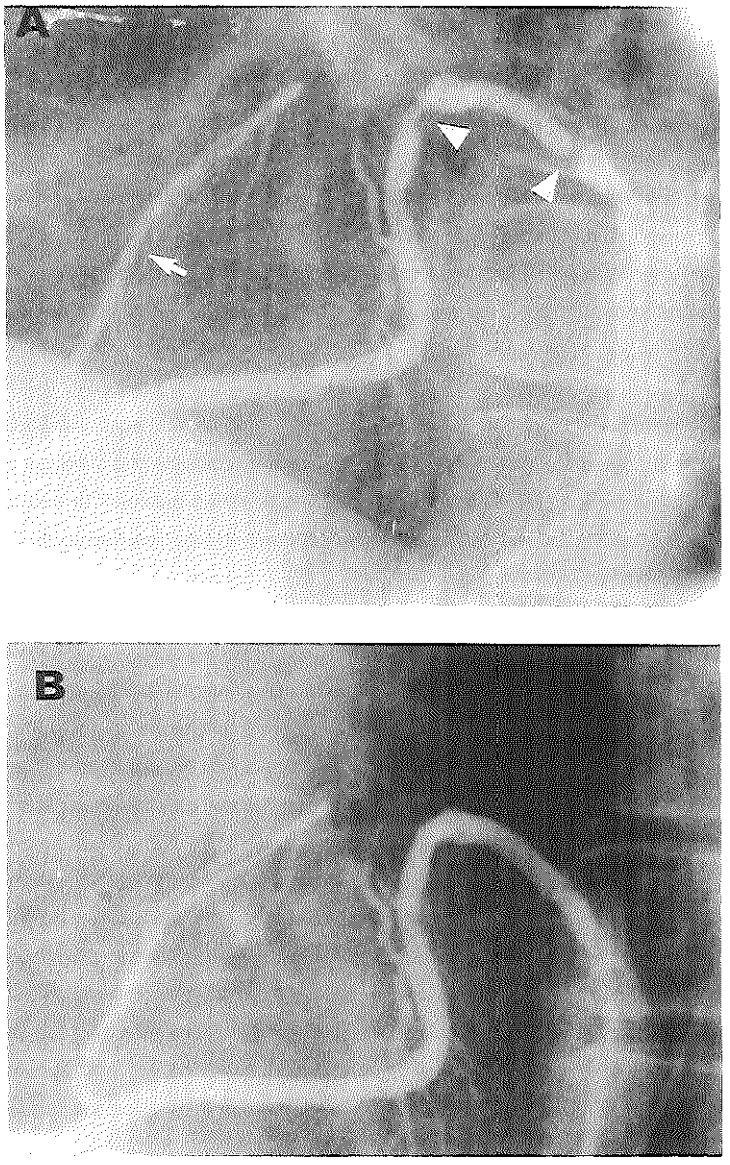

Figure 1. *Jump graft to the left anterior descending artery (arrow), then to the first diagonal branch and then to a marginal branch. A. Two lesions in the jump graft between the diagonal and marginal branches (arrowheads). B. Immediate result post stenting. The distal end of the stent may have extended into the native coronary artery.*

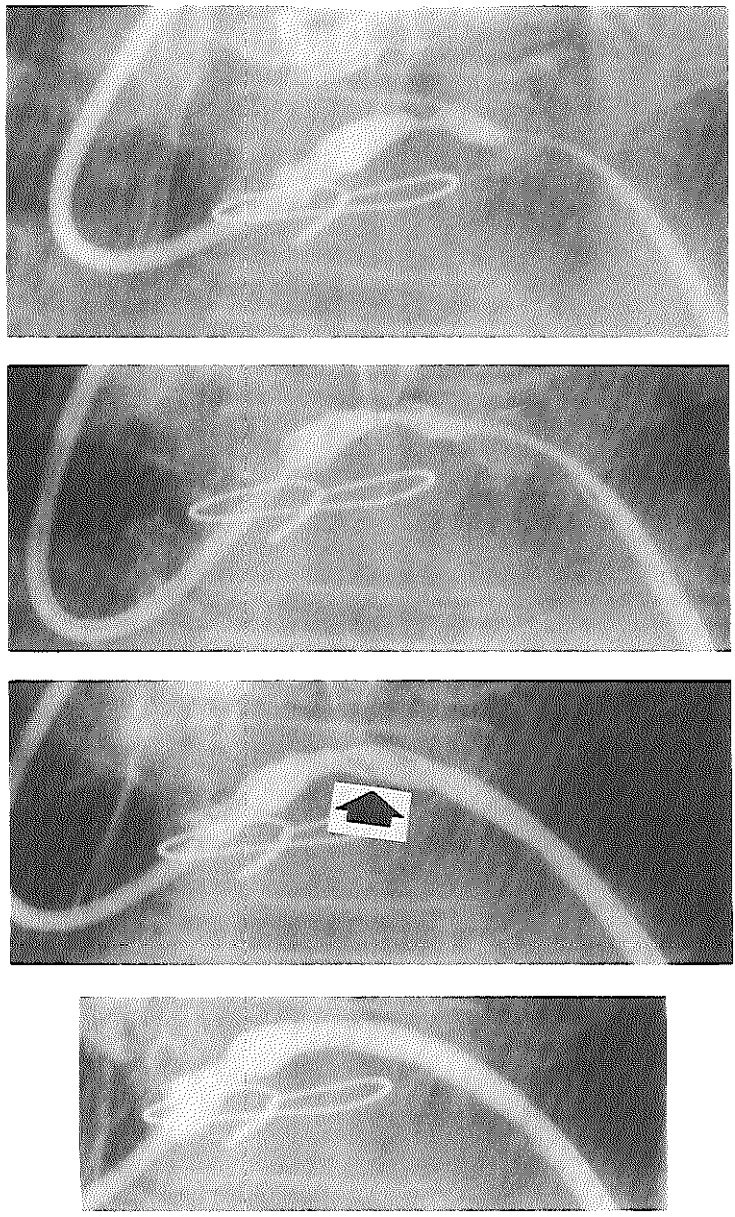

Figure 2. *Top Panel. Two tandem lesions with an aneurysm of the bypass graft located proximal to the first lesion.*
Second Panel. Balloon angioplasty catheter in position across the lesion. Balloon dilatation resulted in a dissection.
Third Panel. After implantation of distal stent, intraluminal flap (arrow) is still evident in proximal lesion.
Lower Panel. After implantation of the second stent in the proximal lesion.

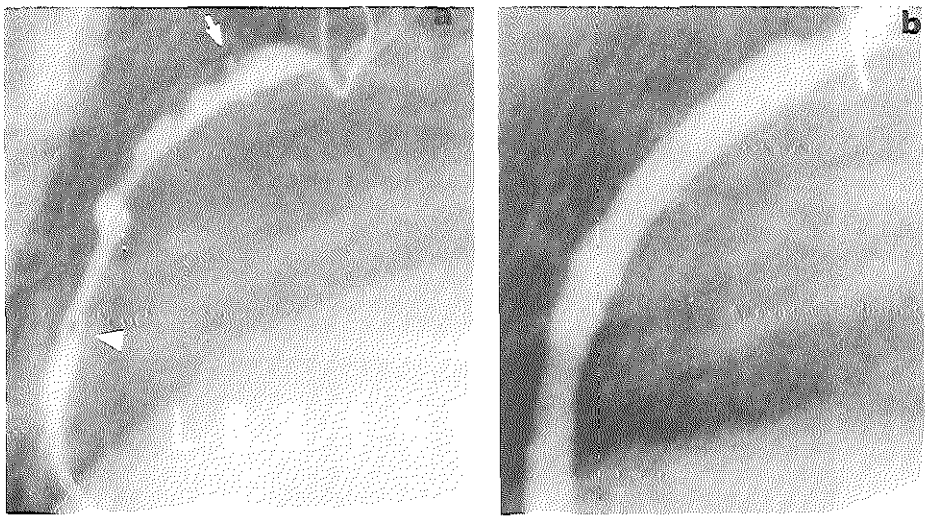

Figure 3A. *Bypass graft to the right coronary artery (7 inch image intensifier). A long segment of the bypass vessel is severely diseased with involvement of the ostium and several complex features including ulceration in the proximal aspect of the graft (arrow) and intraluminal defect in the mid graft (arrowhead).*

Figure 3B. *After placement of 3 stents (5 inch image intensifier).*

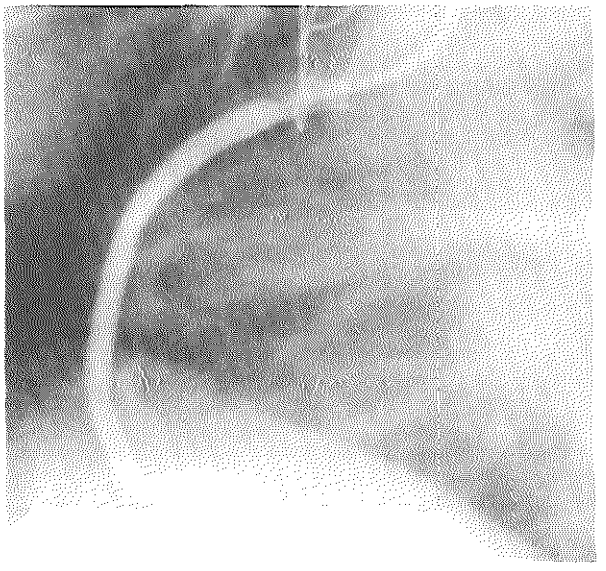

Figure 3C. *Six month angiographic follow-up showing a late excellent result.*

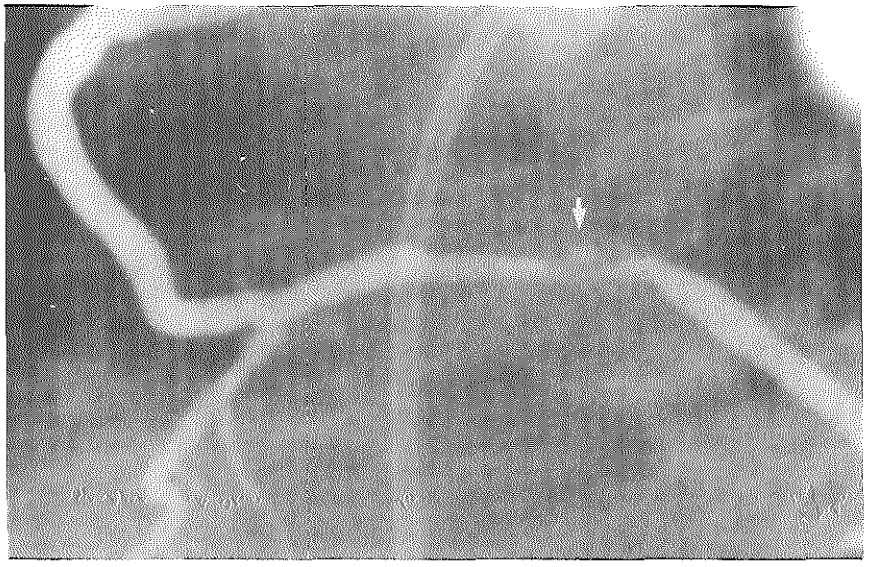

Figure 4A. *Complex lesion with ulceration (arrow) in jump bypass graft prior to stenting.*

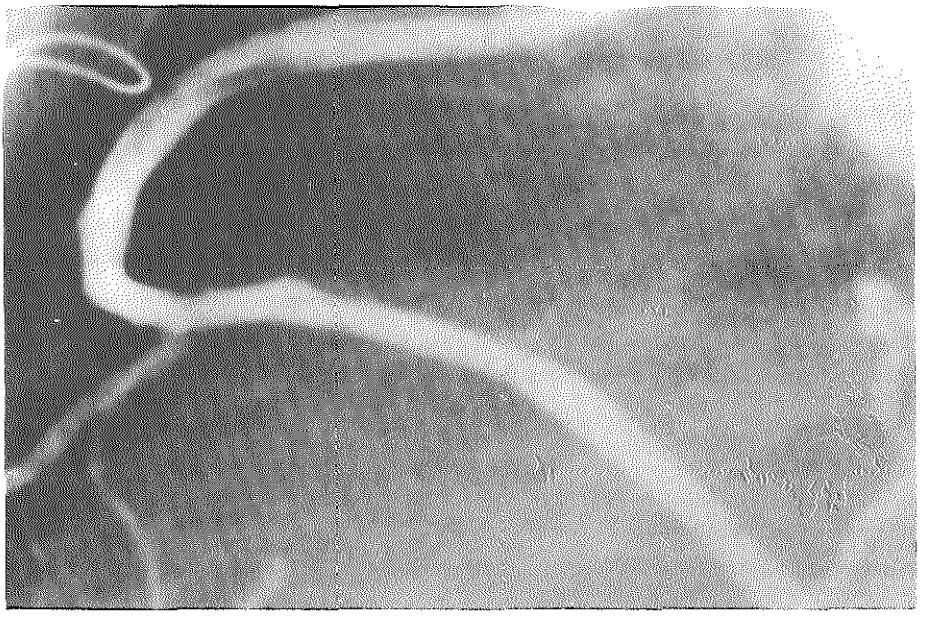

Figure 4B. *Immediate result post stenting.*

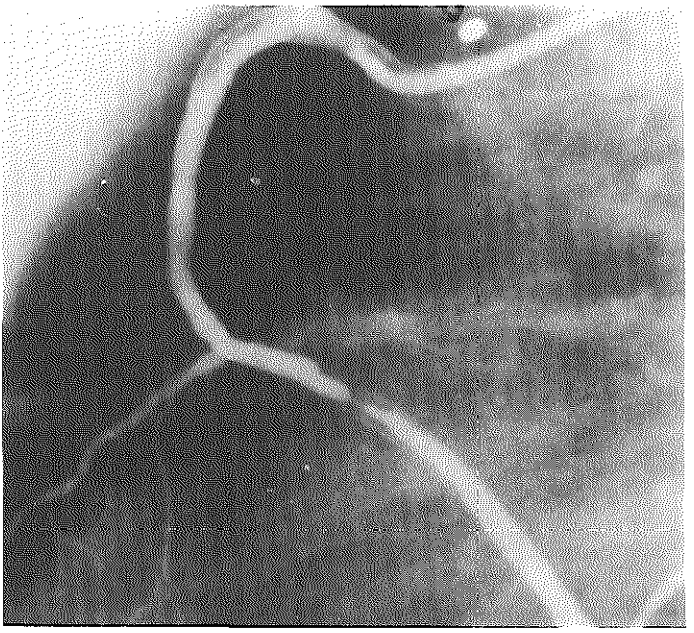

Figure 4C. *Six month angiographic follow-up showing restenosis within the stent. The outline of the stent is faintly visible.*

Restenosis

Two different sets of criteria were applied to determine the restenosis rate. We have found a change in minimal lumen diameter of 0.72 mm or more to be a reliable indicator of angiographic progression of vessel narrowing (23-25). This value takes into account the limitations of coronary angiographic measurements and represents two times the long-term variability for repeat measurements of a coronary obstruction using CAAS. The other criterion for restenosis was an increase of the diameter stenosis from less than 50% after stent implantation to greater than or equal to 50% at follow-up. This criterion was selected according to common clinical practice (29).

RESULTS (Table IV)

Stent implantation procedure

All patients underwent successful stent implantations (DS<50% immediately following placement of the stent). In 2 cases, the initial stent was not optimally positioned and did not cover the entire lesion so that an additional procedure was required to implant another stent to achieve an optimal result. Although no immediate major complications occurred during the procedure, two patients required intracoronary thrombolytics due to distal embolization without subsequent CK elevation. Three other

patients developed minor CK rises (<200 IU).

In hospital complications (Table V)

Acute thrombotic events in the stent occurred in 7 patients (10%). One of these occlusions was related to cessation of anticoagulation treatment. This patient (considered inoperable) developed a severe retroperitoneal haematoma 7 days after implantation of the stent, which necessitated discontinuation of the anticoagulation therapy. Thirty days after implantation, the patient developed an acute myocardial infarction, leading to cardiogenic shock and death. One patient who had the stent implanted during an evolving myocardial infarction, developed an acute thrombotic closure of the stented vessel one day after stent implantation. The resulting myocardial infarction was treated conservatively.

Two other patients, who had unstable angina pectoris and angiographic defects consistent with thrombi, developed acute thrombotic occlusions and myocardial infarctions 7 and 12 days after stent implantation respectively. One of these patients, who was treated conservatively, died suddenly 6 months following implantation, having remained stable with mild angina pectoris. The other patient underwent coronary bypass surgery after emergent reopening of the vessel during rePTCA. The last thrombotic occlusion occurred one day after implantation in one of the patients with stent implantation after recanalization of a chronically occluded graft. This patient was referred for reCABG.

Two patients developed unstable angina pectoris 3 and 10 days after stent implantation, related to an angiographically visible but non-occlusive thrombus. In one patient, this thrombus was related to cessation of anticoagulation 6 days after the procedure due to a Mallory-Weiss syndrome with persistent gastro-intestinal bleeding (30). Due to symptomatic recurrent ischemia, the patient was sent for surgery. The second patient, who had been treated for unstable angina pectoris developed rest angina pectoris one day after implantation of two stents. Angiography revealed a partially occlusive thrombus between the two stents and another stent was placed between the two previous stents. The following day, rest angina pectoris recurred and the patients was treated surgically.

Bleeding complications occurred in 23 patients (33%). Two patients suffered fatal intracranial bleeds, one patient had a retroperitoneal haematoma and two patients developed gastric bleeding. An additional 18 patients had hematomas at puncture sites requiring blood transfusions and 7 of these patients required surgical repair of a false aneurysm. Bleeding complications were associated with a considerably longer hospital stay: 18 days in comparison to 7 days when the postimplantation course was uneventful.

Discharge status

Stent implantation resulted in complete relief of angina pectoris (NYHA I) in 45 patients (64%). Ten patients (14%) still had mild symptoms (NYHA II) after stent implantation, and 5 patients (7%) remained in NYHA class III. Four of these patients were considered inoperable and the fifth was referred for

reoperation.

Long-term follow-up

(i) Angiographic (Table 5)

In fifty-three (90%) of the 59 patients with successful stenting and no major in-hospital complications, follow-up angiography was performed at 4.9 ± 3.4 months. Of the remaining 6 patients without angiographic follow-up, 5 refused a control angiogram, and in another patient the implantation film was technically inadequate for analysis although no significant restenosis was seen at follow-up.

In the overall group the mean minimal luminal diameter increased significantly from 1.4 ± 0.82 to 2.7 ± 0.7 mm ($p<0.001$) and the diameter stenosis decreased significantly from 58 ± 15 % to 24 ± 9 %. However at late follow-up (including occlusions) there was a significant reduction in mean minimal luminal diameter to 1.9 ± 1.1 mm ($p<0.001$) and a significant increase in diameter stenosis to 43 ± 30 % ($p<0.001$).

The incidence of restenosis depended on the definition. Using a criteria of a change in minimal luminal diameter of 0.72 mm, detectable angiographic narrowing occurred within the stent in 25 patients (47%). An increase in diameter stenosis to 50% at follow-up was seen in 21 patients (40%) and immediately adjacent to the stent in 4 patients (7%). Stent occlusion was found in 3 of these patients.

(ii) Clinical

In the group of patients with angiographic restenosis (DS>50% criterion) (n=25), 19 patients experienced recurrence of angina pectoris, necessitating reintervention (rePTCA: n=10, atherectomy: n=2, reCABG: n=7). Three of the patients who underwent surgery died during the postoperative period.

In the group without restenosis (n = 28), 15 patients had recurrence of significant angina pectoris within 1 to 24 months following stent implantation. Ten patients underwent further intervention. In 6 patients a second stent was implanted in either the same or another bypass graft. Three patients underwent PTCA of one or more native vessels and one patient had repeat bypass surgery. The five remaining patients were treated medically. One patient, without significant restenosis at 6 month angiography, died suddenly 500 days after stent implantation.

DISCUSSION

The management of recurrent ischaemia in patients who have had previous bypass surgery presents a serious and growing problem. Symptoms of myocardial ischemia recur or progress in about 5% of patients per year (3,26,27) and after 5 years, up to 25% of vein grafts are occluded and 25% may show stenoses greater than 70% (28). Reoperation is technically more complicated to perform and is generally associated with a higher mortality and morbidity than a primary operation, and achieves symptomatic relief in only 60% to 70% of patients, as compared to the 80% to 90% success after primary operations.

The peri-operative myocardial infarction rate varies among surgical groups between 2.0% and 11.5%. The mortality rate following repeat bypass surgery ranges from 1.2 to 12.5% (1-3). Conventional balloon angioplasty has reported angiographic success rates of 75-100% for bypass grafts (7-13), with complications rates similar to angioplasty in native vessels. However, restenosis appears to occur more frequently with rates as high as 46% reported for proximal sites (24,27).

The majority of our patients were considered high risk for surgery or repeat PTCA. Since all of these patients had severe symptoms in spite of maximal medical therapy, it was decided to try to attempt stenting of the angina-related bypass graft although in some cases it was clear that full relief could not be expected due to diffuse native vessel disease that prohibited additional intervention. Most of the procedures were done without surgical stand-by. Since the introduction of stenting of stenosed saphenous bypass grafts in our institution in 1988, 69 patients were (succesfully) treated by this new intervention compared with only 84 patients who underwent conventional angioplasty for stenosed saphenous bypass grafts during the period 1980-1988. This new treatment modality has significantly expanded our therapeutic options in this particular patient group.

Our results show that stenting bypass grafts is technically feasible with excellent immediate results. Two advantages of the Wallstent® for use in bypass grafts are: (1) the length of the stent can be selected up to 25 mm for long lesions, and (2) the self-expanding property appears to be an effective splint to tack back friable, protuberant atheromatous material and minimize embolization into the native coronary circulation. In 30 patients, the stent was implanted directly without prior balloon dilatation for lesions that looked high risk for embolization. No CK rise was documented in this group of procedures.

Several important lessons emerge from this study. First, the majority of stent occlusions occurred in patients with acute ischemic syndromes (myocardial infarction or unstable angina pectoris with angiographic evidence of thrombi). The combination of thrombi during evolving myocardial infarction and unstable angina and the implantation of intracoronary stents seems to be highly thrombogenic leading to further thrombus formation and acute occlusion of the stent. Therefore, we now carefully select our patients and when the diagnostic angiogram is suggestive for intravascular clots, the patients are treated with intravenous heparin (25000 IV/24 hr) for one week before stent implantation. Although improved patient selection should decrease the occurrence of acute stent closure, it will remain an unpredictable event as evidenced by stent occlusion in 1 patient who was optimally anticoagulated and without the previously described risk factors. Furthermore, the timing of stent occlusion was also unpredictable (between 2 and 12 days) which complicates discharge planning decisions. Second, a meticulous anticoagulation schedule must be followed with frequent monitoring to minimize bleeding complications. As our experience evolved, bleeding and occlusion problems were encountered much less frequently and as a result, sulphinpyrazone was withdrawn due to lack of evidence for its efficacy in the prevention of acute closure. Special care must also be given to femoral arterial sheath insertion and removal since this accounted for the majority of bleeding complications. In particular, removing the sheath >12 hours post implantation was associated with increased vascular complications. During the

last 15 stent implantation procedures, no thrombolytics were administered leading to a considerable decrease in groin bleeding problems. Furthermore, oral coumadins were started the day before stent implantation leading to a quicker optimalization of the oral anticoagulation therapy which made a shorter hospital stay possible.

Stent related restenosis (47%) seems to be comparable to historical studies of conventional angioplasty in venous coronary bypass grafts (4,5,30). However, these comparisons may not be valid since our population consisted of patients who were less than suitable candidates for conventional angioplasty. Earlier reports from Lausanne suggested much lower restenosis rates (9%) in lesions implanted with the Medinvent stent in bypass grafts (17,32). However, these differences may be due to either differences in selection criteria and/or methods of angiographic assessment (quantitative versus visual estimation).

Frequent reintervention in our study group was required due to restenosis or progression of disease in other lesions, a problem similar to conventional angioplasty in bypass grafts. Three recent reports have been published on the late clinical follow-up of patients with conventional angioplasty in bypass grafts. The Thoraxcenter reported that only 41% of patients were alive and event-free (myocardial infarction, repeat CABG, repeat PTCA) at a median follow-up of 2.1 years (4). A review of the overall Dutch experience also showed limited late beneficial results with a two year and five year event free survival of 52% and 26% respectively in 454 bypass patients (5). Webb et al have described a 71% freedom from death, infarction and surgery at 5 years in bypass patients who underwent PTCA at their institution but did not include the 27% incidence of second angioplasty procedures also required in their patient group (31). It is clear that stenting and angioplasty are only short-term solutions and do not affect the underlying problems of progressive graft atherosclerosis and iatrogenically induced restenosis

Conclusion

Patients with severe coronary artery disease and previous bypass surgery who have refractory symptoms due to progression of disease in the bypass graft comprise a difficult challenge to the physician. In patients who are poor surgical risks and unsuitable candidates for balloon angioplasty due to unfavorable anatomy, coronary stenting with the Wallstent® can be performed successfully and offers an alternative therapy. However, stent implantation remains complicated by acute thrombotic occlusion and bleeding complications associated with the intense anticoagulation. The early benefits of stenting may be mitigated by the progression of disease in bypass grafts and iatrogenic induced restenosis. Stenting should be considered a palliative procedure in medically refractory patients with coronary bypass graft disease.

Table I. Study population

Number of patients	69
Number of bypasses	74
Number of lesions	95
Number of stents	136
Ejection fraction (%)	53.9 (26-71)
Risk factors :	
Hypercholesterolemia > 7 mmol/l	26
Hypertension	15
Smokers	46
Diabetes mellitus	14

Table II. Specific Lesion Characteristics

Number of bypasses	74
Age of the bypass graft (mths)	83 (1 - 166)
Mean diameter of the graft (mm)	3.3 (1.6 - 7.0)
Mean minimal lumen diameter (mm)	1.4 (0 - 2.9)
Length of the stenosis (mm)	16.5 (2 - 50)

Table III: Reasons for preferring stent implantation (categories are not mutually exclusive)

-Patients not suitable or high risk for re-CABG	42
Reasons: - high risk/benefit profile	28
- unfavourable coronary vessel anatomy	4
- poor left ventricular function (EF<35%)	6
- concurrent noncardiac risk factors	4
- Patients considered high risk for conventional PTCA	39
Reasons: - long lesions (>15 mm)	32
- tandem lesions	23
- lesions containing:	
ulcers	25
dissections	17
clot	18
- diffusely diseased bypass graft	34
- eccentric lesins	53

Table IV. Stent implantation data

Number of stents	136
Mean diameter of stent (mm)	4.3 (3.5 - 6.0)
Position	
ostial	7
shaft	127
distal anastomosis	4
Procedure Type	
Single Stent-Single Lesion	30
Multiple Stent-Single Lesion	90
Multiple Stent-Multiple Lesion	16

Table V. Clinical follow-up

IN HOSPITAL COMPLICATIONS	
- Acute thrombotic complications	7
- Death	1
- MI	5
- Acute surgery	4
- Bleeding problems	23
- Death	2 (intracranial bleed)
- Recurrence AP	5
- re-CABG	1
PATIENTS WITH ANGIOGRAPHIC FOLLOW UP	53
- Angiographic Restenosis (DS > 50%)	25
- re-PTCA	10
- Atherectomy	2
- re-CABG	7
- AMI	2
- Death	3 (after CABG)
-Patients without angiographic restenosis	26
15 progression of disease at another site	
- Native	11
- Bypass	4
- re-stent	6
- re-PTCA	3
- re-CABG	1
- AMI	2
- Death	1
LATE DEATH	1

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Chapter 9

Directional atherectomy for treatment of restenosis within coronary stents: Clinical, angiographic and histologic results

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(submitted)

Abstract

In 9 patients (10 procedures) directional atherectomy was performed 82-1179 days for restenosis within a stented coronary segment. The tissue was subsequently studied for the presence of intimal hyperplasia, extent of proliferation and cell density. A control (non-stented) group consisted of 13 patients who had restenosis tissue removed 14-597 days following an initial procedure (PTCA, atherectomy or laser).

The atherectomy procedures within the stent were all technically successful. In one case a small fragment of the stent was removed. For the entire group, the post atherectomy result was similar to the initial result after stenting (2.31 ± 0.38 mm versus 2.44 ± 0.35 mm). Five patients had follow-up 44-131 days after atherectomy, and three of the patients had diameter stenosis > 50%. Three patients required reintervention (surgery, n=2; repeat atherectomy and then laser angioplasty, n=1).

Intimal hyperplasia was identified in 80% of the specimens after stenting and in 77% after PTCA or atherectomy. No differences were seen in tissue removed from stenting versus PTCA/atherectomy. In three stented patients (47-143 days after stenting) 70-76% of the intimal cells showed morphologic features of a contractile phenotype by electron microscopy. Evidence of ongoing proliferation (PCNA antibody studies) were absent in all specimens studied. Although large individual variability was present in the maximal cell density of the intimal hyperplasia, there was a trend toward a reduction in cell density over time.

Atherectomy can be safely performed for restenosis in stented coronary arteries with excellent initial results. However, restenosis continues to limit the late effectiveness. Intimal hyperplasia is a non-specific response to injury regardless of the method and accounts for about 80% of cases of restenosis. This preliminary study suggests that smooth muscle cell proliferation and phenotypic modulation towards a contractile phenotype are early events and largely completed by the time of clinical presentation of restenosis (ie. < 2months). Cellularity results suggest that lesions may be predominantly cellular, matrix or a combination at a particular time after a coronary procedure.

INTRODUCTION

Restenosis remains the major limitation of percutaneous transluminal coronary angioplasty (PTCA), occurring in 20-40% of patients within the first 6 months after angioplasty (1). The implantation of stents in coronary arteries or saphenous vein bypass grafts as an adjunct or alternative to PTCA was initially proposed to prevent late restenosis (2). However restenosis has now been documented in a significant number of patients in the first 6 months following stenting (3,4). The optimal method to prevent restenosis or to treat its occurrence (or recurrences) after PTCA or coronary stenting is unknown. No pharmacological treatment has been consistently successful in reducing restenosis rates after PTCA (5). Although restenosis occurs with the use of mechanical devices other than PTCA, no randomized trials have yet been reported to determine if more favorable restenosis rates result from their use. Directional atherectomy is one of these alternative mechanical devices for nonoperative coronary vascularization. In selected patients excellent post procedural results have been documented (6). Furthermore, since the tissue can be removed, it offers a unique opportunity to study the histological features of the restenosis tissue.

In the past two years, we have collected data from 10 procedures performed for restenosis within a stented coronary segment that were treated with directional atherectomy. The purpose of this study was twofold: 1) to determine the feasibility, safety and late results of directional atherectomy for treatment of restenosis within coronary stents and 2) to assess the tissue removed from the restenotic lesion that caused the narrowing within these stents. Although restenosis after PTCA has been characterized by proliferating smooth muscle cells associated with extracellular matrix formation, we were particularly interested if differences existed in restenosis after stenting. In addition, since the temporal changes in the histological pattern following PTCA are largely unknown and have been studied in only a limited number of patients (7), we wanted to study the proliferation rates and cell density of restenosis tissue removed at specific intervals of time to better characterize the development of restenosis. For the histological studies, we have compared tissue retrieved by coronary atherectomy in 9 patients with restenosis in stented arteries with tissue obtained from 13 patients with restenosis after PTCA or previous atherectomy without adjunct stenting.

METHODS

The stent study population consisted of 9 patients who underwent 10 separate atherectomy procedures within the stent. Five of the patients were treated in Rotterdam, 2 patients in Belgium, 1 patient in United States and 1 patient in Toulouse, France. The clinical characteristics are presented in Table 1. Five of the procedures were performed in stents placed in bypass grafts and the other 4 stents were implanted in native vessels (Table 2). Six of the stented vessels contained the Wallstent^{R} (Schneider, Zurich) which is a self expandable stainless steel woven mesh stent (3,4). Two patients had

been implanted with a Palmaz-Schatz™ stent (Johnson and Johnson, Warren, New Jersey) which is a balloon expandable stainless steel tubular stent (8). One patient had received a Wiktor™ stent (Medtronic, Minneapolis), a tantalum balloon expandable stent with a helical coil design (9). Five of the patients were stented for primary lesions. The remaining 4 patients were originally stented for restenosis after PTCA. Two of these patients (Patient 4 and 9) had multiple restenoses and one of these patients (Patient 4) underwent a second atherectomy procedure within the stent for a restenosis recurrence after the initial atherectomy. Atherectomy was performed within the narrowed stent 82-1179 days post stenting. Three of the patients had separate PTCA procedures for stent-related problems prior to the atherectomy. Patient 1 initially underwent PTCA for restenosis 97 days after stenting and then required an atherectomy procedure 47 days later for a second restenosis within the stent. Patient 3 underwent balloon angioplasty for restenosis 210 days after stent implantation and then atherectomy 156 days later (366 days after stenting). Due to restenosis, a second atherectomy procedure was done 96 days after the first atherectomy (462 days after stenting). Patient 8 had a symptomatic acute occlusion five days after stenting. After recanalization with intracoronary streptokinase and PTCA, he had an uneventful recovery until he experience recurrence of angina 5 months later due to restenosis within the stent. Patient 9 received a second stent for a different lesion in the bypass graft 570 days after the first stent. A lesion subsequently developed in the initial stent and was treated by atherectomy 1179 days after the first stent implantation (609 days after the second stent).

For the histological evaluation of the tissue, we selected a control group which consisted of all patients in the Thoraxcenter experience who underwent an atherectomy procedure for restenosis after PTCA, atherectomy or laser (n=13) (Table 5). This group consisted of 11 men and 2 women, and the ages ranged form 40-71. The interval of time between the most recent intervention and atherectomy for restenosis ranged from 4-597 days.

Angiographic Analysis

All cineangiograms were analyzed using the computer assisted cardiovascular angiography analysis system (CAAS) which has previously been discussed in detail (10). The important steps will be briefly described. Any area of size of 6.9 X 6.9 mm in a selected cineframe (overall dimensions 18 X 24 mm) encompassing the desired arterial segment can be digitized by a high resolution CCD-camera with a resolution of 512 X 512 pixels and 8 bits of gray level. Contours of the the desired segment are determined automatically, based on the weighted sum of the first and second derivative functions applied to the digitized brightness information along scanlines perpendicular to the local centerline of the desired segment. A computer-derived estimation of the original dimension at the site of the narrowing is used to define the interpolated reference diameter. This technique is based on a computer-derived estimation of the original diameter values over the analysed region (assuming there was no narrowing present) according to the diameter function. The absolute diameter of the stenosis as well as the reference

diameter are measured by the computer which uses the known outside diameter of the perspex model as a calibration factor.

Tissue Analysis

Following extraction of the tissue with the Simpson coronary atherocath^R, the specimens were carefully removed from the housing chamber of the catheter, washed with 0.9% saline and cut into pieces of approximately 1 mm by 1-2 mm. Representative pieces were fixed in 10% buffered formalin for light microscopy studies. The specimens were processed according to standard procedures and then paraffin sections were stained with haematoxylin and azophloxine, and with Van Gieson. Three to five slides were prepared at various levels through the paraffin block. The tissue was specifically assessed for the presence of intimal hyperplasia and atherosclerotic plaques according to the definitions of Johnson et al (11). Intimal hyperplasia was defined as highly cellular tissue consisting of randomly arranged stellate and spindle cells in an abundant, collagen containing extracellular matrix. Atherosclerotic plaques consisted of dense fibrous tissue with abundant collagen, scattered fibroblasts and occasional mononuclear cells, including lymphocytes, and macrophages/foam cells.

Immunohistochemical studies

In deparaffinized sections, immunostaining was performed with monoclonal antibodies directed against alpha-smooth muscle cell actin (Sigma, St. Louis, Missouri) using an indirect conjugated peroxidase procedure. Proliferating cells were identified immunoctyochemically using a monoclonal mouse anti-human proliferating cell nuclear antigen (PCNA) antibody (DAKO-PCNA, PC10, Glostrup, Denmark). This antigen is a DNA polymerase auxiliary protein and is expressed during G1, S (DNA synthesis), and G2 phases of the cell cycle (12-14) but not in the quiescent G_o phase.

Electron Microscopy

Representative pieces were fixed in a solution of glutaraldehyde-formaldehyde (4CF-1G). Postfixation was done with OsO_4. The specimens were then embedded in epon and ultrathin sections were stained with uranyl acetate and lead citrate. All specimens contained smooth muscle cells with abundant extracellular matrix. Cells were assessed as either contractile or synthetic type smooth muscle cells based on the following morphologic features. The synthetic cells were characterized by an abundant cytoplasmic organelles including endoplasmic reticulum, Golgi apparatus and ribosomes, and by peripheral location of myofilaments (Figure 1a). The cytoplasm of the contractile cells consisted mostly of myofilaments and a few mitochondria (Figure 1b). Cells were counted in multiple fields (at least 150 cells in total) and classified according to these criteria into the two phenotypes.

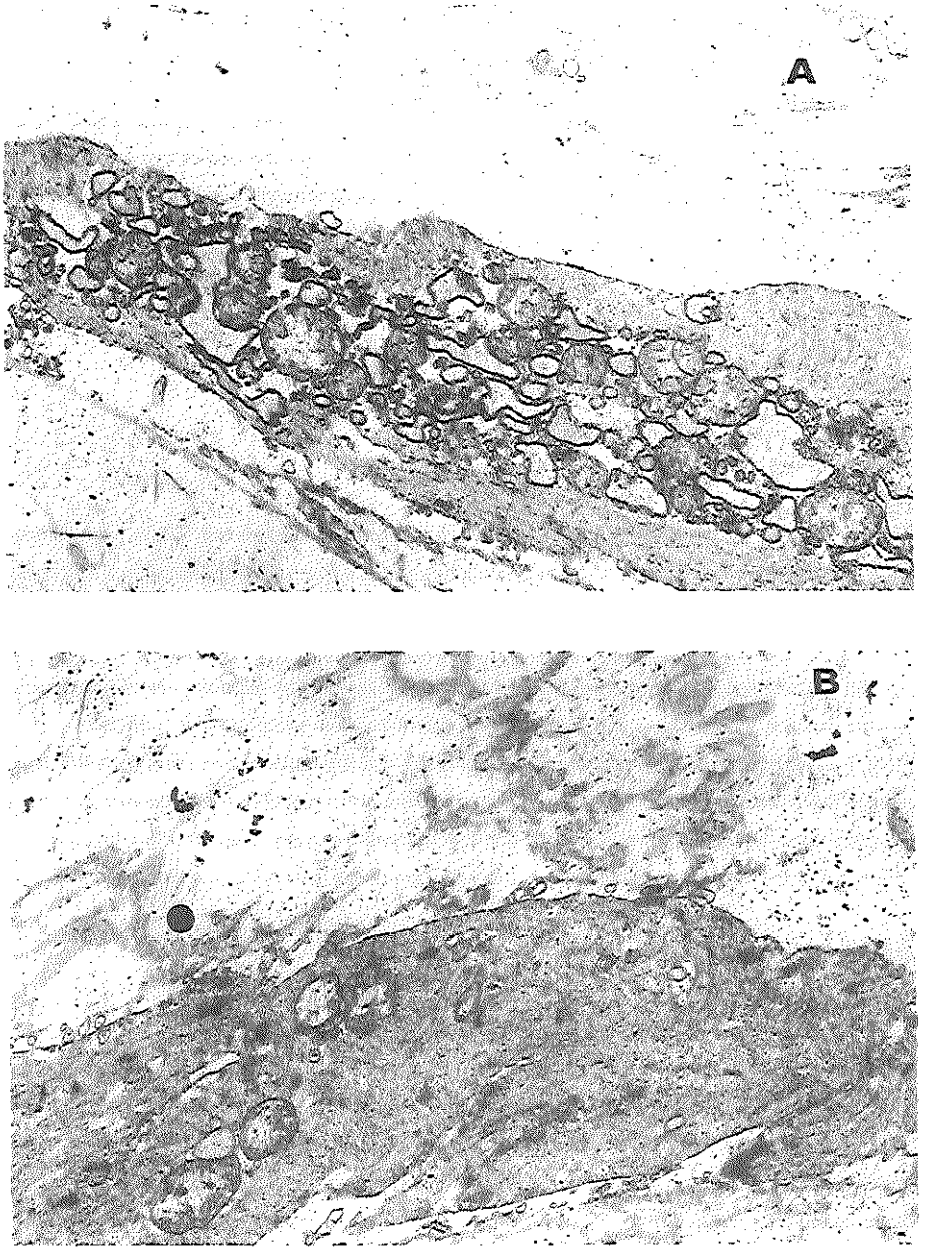

Figure 1. *Transmission electron micrograph showing A) a smooth muscle cell with the synthetic phenotype. There are abundant cytoplasmic organelles including endoplasmic reticulum, Golgi apparatus, and ribosomes. Only a few myofilaments are present and are located in the periphery of the cell. B) Contractile smooth muscle cell cytoplasm consists mostly of myofilaments and a few mitochondria.*

Cell Density of Intimal Hyperplasia

In haematoxylin and azophloxine stained sections, areas of intimal hyperplasia were identified and cell number was assessed in several fields by a computerized morphometry system (IBAS, Kontron, Oberkochen, Germany). The maximum value recorded was used for the determination of cell density which was expressed as cell number/ mm^2 intimal tissue. Specimens without intimal hyperplasia were excluded from this measurement since this part of the study was specifically designed to look for temporal changes in cellularity occurring in intimal hyperplasia formed in response to the coronary procedure.

RESULTS

Clinical

All atherectomy procedures were technically successful (residual stenosis < 50% with retrieval of tissue) and there were no procedural complications other than a transient ischemic attack that occurred during a PTCA of a separate lesion in one patient. The only technical problem occurred with the Wiktor™ stent. Following the procedure, the configuration of the stent was disrupted although no complications ensued. Tiny fragments of the tantalum wire were observed in the atherectomy material. All of the patients experienced immediate improvement in their symptoms. At late follow-up (4-15 months), Patient 2 died following bypass surgery for restenosis after atherectomy and Patient 5 died due to endstage renal failure. Two other patients required additional interventions for recurrence of symptoms due to restenosis after atherectomy. Patient 1 underwent bypass surgery 6 months after the atherectomy and Patient 4 was treated with excimer laser therapy. Five of the patients remained in NYHA Class I-II.

Quantitative Angiography (Table 3)

Immediately after placement of the stent there was an overall significant increase in the minimal luminal diameter and a significant decrease in the percentage of the diameter with stenosis (changing from a mean [±SD] of 1.12±0.36 to 2.44±0.35 mm and from 63±9% to 21±10%, respectively; $p<0.001$). However, at follow-up prior to the atherectomy, all of the lesions had deteriorated to an overall value of 0.99±0.24 mm and 64±7%, respectively. The immediate result after atherectomy was similar to the acute stenting results (2.31±0.28 mm, 27±10%). Late follow-up after atherectomy was only done in 5 of the lesions, with significant deterioration (loss of ≥0.72 mm) occurring in three lesions. An example of the angiographic appearance of the lesion pre and immediately post stenting, at follow-up (pre atherectomy) and post atherectomy is shown in Figure 2.

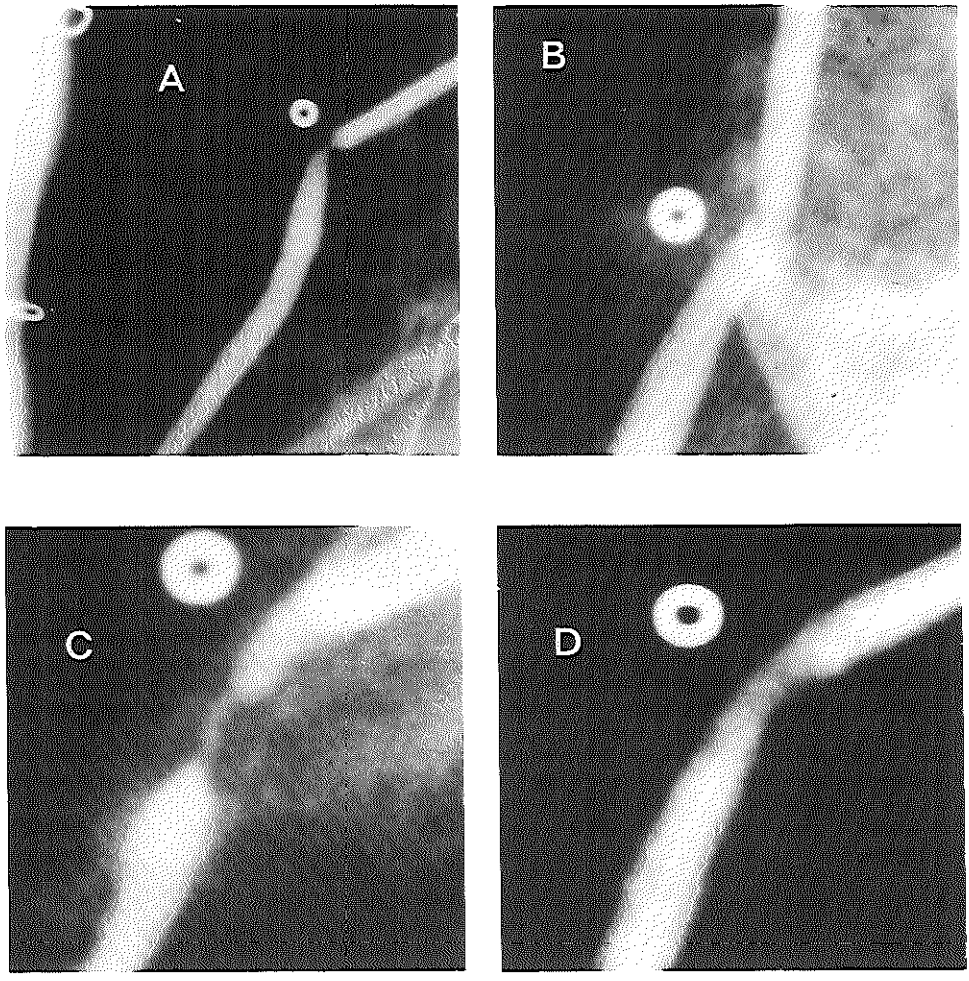

Figure 2a. *Stenosis in proximal bypass graft prior to stenting.* **Figure 2b.** *Immediate result after stenting.* **Figure 2c.** *Restenosis in stent distal to original site of stenosis.* **Figure 2d.** *Immediate result after atherectomy within the stent.*

Histology

(i) After Stenting (Table 4)

The characteristic feature in tissue obtained in 8 of the lesions was intimal hyperplasia defined as a proliferative cellular response associated with a matrix of loose connective tissue. The area of intimal hyperplasia was typically sharply demarcated from the underlying sclerotic plaque. However the cellularity, amount of collagen, and extracellular matrix of the intimal hyperplasia varied between patients (Figures 3 and 4).

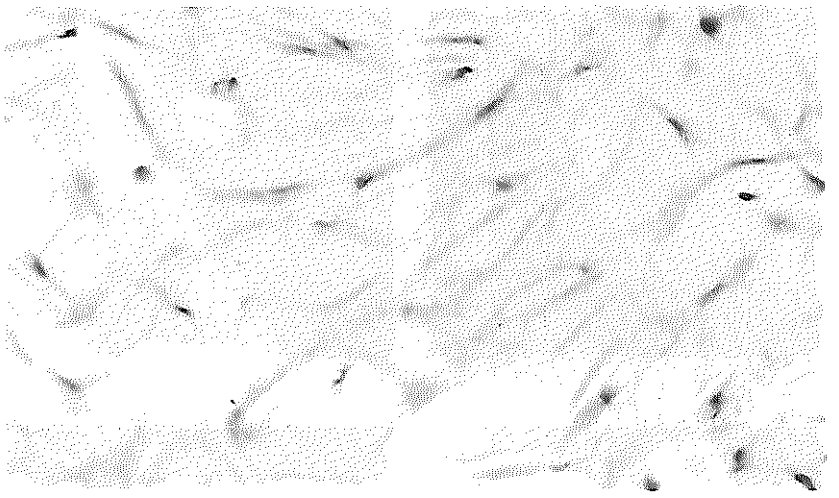

Figure 3. *Haemotoxylin-azofluoxine stained section of tissue removed from a stent 89 days after stenting. The section has the typical appearance of intimal hyperplasia (highly cellular tissue consisting of randomly arranged stellate and spindle cells in a loose extracellular matrix). (original magnification 25x).*

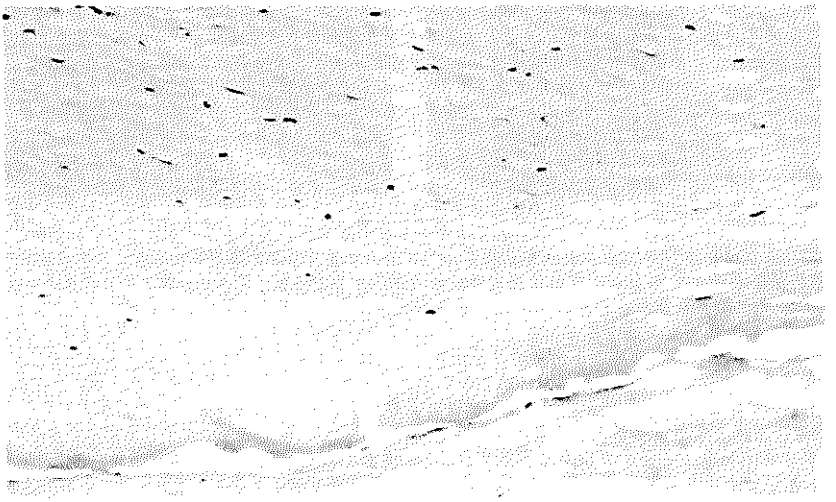

Figure 4. *Haemotoxylin-azofluoxine stained section of tissue removed from a stent 156 days after stenting. No intimal hyperplasia was present. The tissue consisted of a few cells embedded in an abundant, collagen containing extracellular matrix. (original magnification 10x).*

In eight of the lesions, the main cell type within the lesions was identified as smooth muscle cells based on presence of SMC specific alpha actin. Specific staining for endothelial cells and macrophages was negative in two specimens tested although lymphocytes were identified in tissue from Patient 3. No giant cells as evidence of a foreign body reaction were identified in any tissue specimen. In three of the specimens prominent capillary ingrowth was evident. In two specimens, the internal elastic lamina and adjacent media were identified. No evidence of adventitia was recovered (Figure 5).

Ultrastructural studies in three of the stented patients (Patients 1, 6 and 8) showed that the majority (70-76%) of intimal cells were contractile in morphology. No differences could be appreciated at the different time intervals.

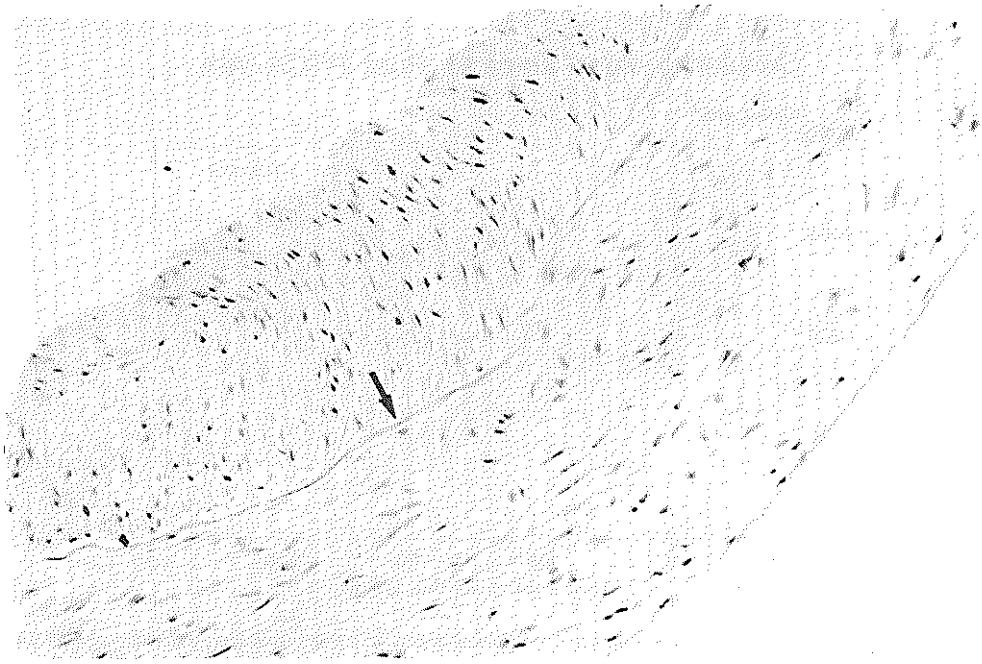

Figure 5. *Haemotoxylin-azofluoxine stained section of tissue removed from a stent 82 days after stenting. The presence of the media is indicated by the internal elastic lamina (arrow) and the typical architecture of the smooth muscle cells in the media. (original magnification 10x).*

(ii) After PTCA/Atherectomy (Table 5)

In the control PTCA/atherectomy group, the histologic appearance of the tissue was indistinguishable from the stent tissue. Intimal hyperplasia was evident in 10 of the thirteen specimens, and again various stages of cellularity were evident. Media was obtained in 3 of the specimens (22%). No evidence of

adventitia was recovered.

Proliferation studies (Table 5 and 6)

In all specimens studied, no cells could be identified that reacted with the antibody to PCNA.

Cell Density

The maximal cell density of the intimal hyperplasia in both the stent and control groups is shown in Figure 6. Although large individual variability was present, there was a trend towards a reduction in cell density of the intimal hyperplasia over time.

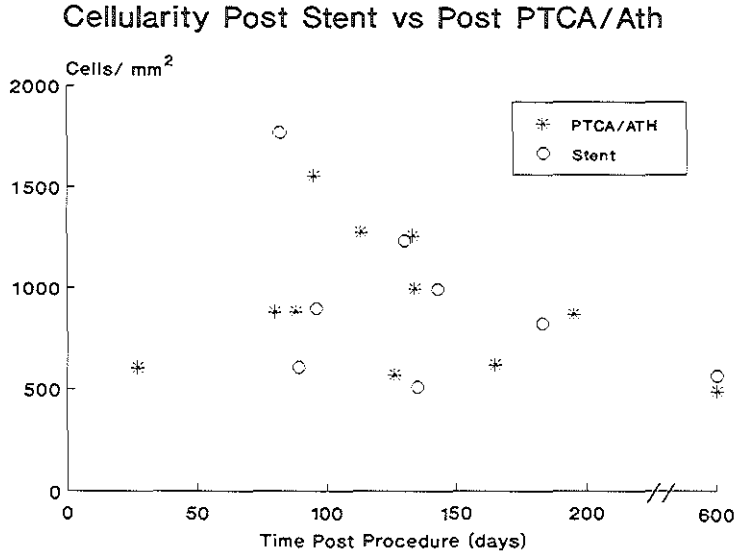

Figure 6. *Maximal cell density (cell number/ mm^2) in restenosis lesions following stenting, and PTCA or atherectomy. The x axis represents the number of days after the procedure. Only restenosis lesions with intimal hyperplasia were evaluated. There is a trend towards decreasing cellularity over time in these lesions although considerable individual variability is evident.*

DISCUSSION

Restenosis persists as an important limitation to all forms of non-operative coronary revascularization, despite increasingly more complex forms of interventions such as stenting, atherectomy and laser-assisted therapy. It remains to be established whether any mechanical method can effectively treat (and

prevent recurrent) restenosis after coronary balloon angioplasty or stenting. We studied the efficacy of directional atherectomy in 10 cases as a procedure to prevent recurrence of restenosis. This study illustrates in a limited number of patients that directional atherectomy can be safely performed within a coronary stent and provide immediate results comparable to the initial stenting procedure. In fact, atherectomy may be a safer procedure in stented than in nonstented vessels since the wires appear to limit the depth of the cutter into the vascular wall and thus reduce the possibility of perforation. However it is still possible to remove media (as in Patients 3 and 6) either between the stent wires or if the stent wire has penetrated the internal elastic lamina. Restenosis occurred in both cases of medial resection. The problem of removing or disrupting part of the stent should be particularly of concern when the restenosis occurs immediately proximal to the lesion and the cutter can abut against the proximal part of the stent. Although the immediate results of atherectomy were excellent, the recurrence of clinical restenosis in three of the patients (33%) in the first 6 months after the atherectomy procedure emphasizes that atherectomy alone will not prevent the restenosis problem.

Histological evaluation of the tissue retrieved from restenosis lesions (after stenting, PTCA, atherectomy or laser) confirms the findings of previous studies that 1) in atherectomy specimens, intimal hyperplasia is the characteristic feature in 75-80% of cases, and that the remaining 20-25% of cases contain only atherosclerotic plaque material without the features of intimal hyperplasia (11) and 2) smooth muscle cells are the predominant cell type found in restenosis lesions (15-17). It is unclear whether the absence of intimal hyperplasia in restenosis lesions is due to a sampling error by the atherectomy catheter or due to another mechanism of restenosis such as elastic recoil or inadequate inital dilatation. If larger studies confirm this observation, the clinical importance is that restenosis interventional trials (pharmacological or mechanical) with the intention to prevent smooth muscle cell proliferation and the formation of intimal hyperplasia, can only potentially affect approximately 75% of the restenosis population at risk. Future study designs may consider this in the determination of sample size for restenosis trials. In addition our study also illustrates that intimal hyperplasia predominates in restenosis tissue, regardless of the initiating procedure, with no unique features attributable to senting in general or to a particular type of stent. This underscores the fact that intimal hyperplasia is a nonspecific response to vascular injury regardless of the method of damage (18,19).

The temporal sequence of events in the formation of intimal hyperplasia following coronary intervention remain largely unknown. Results from our study suggest that:

1) Smooth muscle cell proliferation is an early event, and barely detectable 2 months after the procedure. To date, there is no data on the cell proliferation rates in humans following balloon angioplasty although the use of cyclin immunocytochemistry to label proliferating cells in human de-novo atherosclerotic plaques has previously shown a labeling index ranging from less than 1% to greater than 4% (20,21). Our results, showing no proliferative activity in the smooth muscle cells 82 days to 700 days post stenting, suggest that smooth muscle cell proliferation is an early and limited process after vascular injury in humans. This is similar to the results following vascular balloon denudation in animals in which

SMC proliferation is first observed 48 hours after vascular injury and peak proliferation occurs at about 1 week which is followed by a rapid decline reaching base-line values by a month after the vessel injury (22). Due to the limited period of SMC proliferation early after coronary angioplasty, pharmacological agents designed to reduce proliferation may only be required in the first two months after the procedure rather than the six months usually prescribed.

2) The vast majority of smooth muscle cells modulate towards the contractile phenotype early after the procedure. Therefore only a relatively small percentage of the smooth muscle cells (ie. those with the synthetic phenotype) appear to be responsible for the synthesis of extracellular matrix proteins since in-vitro studies have shown that the production of proteoglycans and collagen is 5 fold and 26-45 fold higher, respectively, in the synthetic phenotype (23,24). In our study, synthetic type smooth muscle cells only comprised 24-30% of the overall smooth muscle cells in the three patients who had atherectomy performed 135-205 days post stenting. In contrast, Nobuyoshi et al identified "synthetic" type smooth muscle cells as the predominant cell type in the first 6 months and thereafter, the "contractile" type smooth muscle cell was dominant (7). This earlier predominance of contractile smooth muscle cells in our study may be due to differences in methods of assessment (electron microscopy versus less reliable light microscopic features in Noboyoshi's series) or possibly related to differences in procedures (stenting versus PTCA alone). Interestingly, in a balloon-injury model in rats, Kocher et al observed a similar phenotypic change (ie. to a contractile type) to our study in lesions 75 days after injury, based on the ratio of smooth muscle to nonmuscle actins that had returned to levels of normal medial (contractile) SMCs (25).

3) Lesion cellularity decreases as a function of time but with a large interindividual variability. As a consequence, lesions may be predominantly cellular, matrix or a combination at a particular time after a coronary procedure. Restenosis has been regarded as a process that is largely completed by 6 months after a procedure. Although cellular proliferation and matrix synthesis are recognized as the components of the restenosis lesion, the remodelling of the vessel wall after vessel injury is not understood and the relative contribution (and possibly the preeminent role) of the matrix components (proteoglycans and collagen) has not been appreciated. A temporal relationship between the cellularity of the intimal hyperplasia lesions appears to exist (although large individual variability is present). Since cellular proliferation appears to be an early event, the cellularity of the lesion is primarily related to the amount of synthesized matrix. The wide range of cell density at a particular interval of time may be related to either inherent biological variability or possibly sampling error. The total amount of matrix present at a particular time is related to the synthesis and resorption of the particular component. The turnover of proteoglycans is unknown although the limited data shows low collagen and elastin turnover in experimental models of hypertension (26). The individual variability in cell density emphasizes the differential importance of matrix deposition in individual lesions. Further studies to characterize the composition and extent of the matrix synthesis during remodelling of the vessel after atherectomy, stenting or PTCA should contribute to a better understanding of the restenosis process. Furthermore,

agents that interfere with matrix synthesis are a relatively untested form of therapy and may be synergistic to pharmacologic approaches to limit smooth muscle cell proliferation which to date have not affected the rate of restenosis.

Study Limitation

This study is primarily limited by the relatively small amount of tissue extracted by the atherectomy catheter which causes a potential sampling bias error. In particular, the device may not have removed the region of intimal hyperplasia containing the highest cell density or high cell proliferation or possibly even may have completely missed areas of intimal hyperplasia in specimens that only showed old atheroma. Therefore the findings from this study with respect to the remodelling of the lesion over time should be confirmed in larger studies.

Acknowledgement
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Table 1. Clinical Characteristics

Patient	Age	Sex	Previous MI	Previous CABG	Smoker	Hypercholesterolemia	DM	Hypertension
1	56	M	1986	23/9/82	-	-	-	+
2	58	M	-	-	-	+	-	-
3	41	M	-	-	-	-	-	+
4b	55	M	Inferior,1977	1977/1983	-	+	+	-
5	64	M	-	Sept 1986/Dec 1987	+	+	+	+
6	67	F	Posterior NQMI	26/3/86	-	+	-	-
7	67	M	-	17/1/89				
4a	55	M	Inferior, 1977	1977/1983	-	+	+	-
8	44	M	Inferior, 1989	No	-	+	-	-
9	76	M	Inferior, 1974	1974	-	+	-	-

Table 2. Stent Characteristics

Patient	Stent Vessel	Stent Type	Stent Diameter (mm)	Reason For Stent	Time to Atherectomy	Present Status (NYHA Class)
1	CABG	Wallstent	3.5	Primary	47 days (144)	Surgery for restenosis
2	LAD	Palmaz-Schatz	3.0	Restenosis	82 days	Surgery for restenosis
3	LAD	Palmaz-Schatz	3.5	Primary	89 days	I
4b	LAD	Wallstent	3.5	Restenosis	96 days (462)	Laser angioplasty
5	Circumflex	Wallstent	3.5	Primary	130 days	
6	CABG	Wallstent	4.0	Primary	135 days	I
7	CABG	Wallstent	4.0	Primary	143 days	II
4a	CABG	Wallstent	3.5	Restenosis (x4)	156 days (366)	see above
8	RCA	Wiktor	3.5	Restenosis	183 days	I
9	CABG	Wallstent	5.0	Restenosis (x2)	1179 days (609)	

Table 3. Anglographic Results

		Pre Stent		Post Stent		FU-S	Stent Follow-Up		Post Atherectomy		Ath Follow-Up		FU-Ath
Patient	Reference Diameter (mm)	MLD (mm)	DS (%)	MLD (mm)	DS (%)	(days)	MLD (mm)	DS (%)	MLD (mm)	DS (%)	MLD (mm)	DS (%)	(days)
1	2.37	1.07	56	1.95	22	49 (144)	0.91	62	2.58	8	0.63	74	131
2	2.84	1.15	59	2.67	5	82	1.19	56	2.26	24	1.89	39	14
3	3.14	1.84	49	2.84	14	89	1.34	58	2.18	32	1.34	58	44
4b	3.1					96	0.90	58	2.33	47	NA		
5	3.1	1.1	60	2.1	19	130	1.30	59	2.37	25			
6	2.9	0.6	72	2.0	38	135	0.63	78	1.77	32	2.46	22	186
7	2.25	0.75	78	2.55	30	143	0.70	69	2.1	29	not done		
4a	3.1	1.2	61	2.8	15	156 (366)	1.03	66	2.81	21	0.9	58	96
8	3.33	0.96	71	2.36	28	183	1.11	62	2.34	29	2.37	20	75
9	2.75	1.40	64	2.71	21	1179	0.81	71	2.4	22	not done		
Mean	2.89	1.12	63	2.44	21		0.99	64	2.31	27	1.79	39	
(±SD)	(0.35)	(0.36)	(9)	(0.35)	(10)		(0.24)	(7)	(0.28)	(10)	(0.67)	(19)	

Table 4. Histology Results

Patient	Duration Post Procedure	Intimal Hyperplasia	Media	Adventitia	Actin	PCNA
1	47 days (144)	-	-	-	+ +	-
2	82 days	+	+	-	NA	NA
3	89 days	+	-	-	+ +	-
4b	96 days (462)	+	-	-	+ +	-
5	130 days	+	+	-	NA	NA
6	135 days	+	-	-	+ +	-
7	143 days	+	-	-	+ +	NA
4a	156 days (366)	-	-	-	+ +	-
8	183 days	+	-	-	+ +	-
9	1179 days (609)	+	-	-	+ +	-

Table 5. Histology Results of Restenosis after PTCA/Atherectomy

Patient	Age	Sex	Vessel	Procedure	Duration Post Procedure	Intimal Hyperplasia	Media	Adventitia	PCNA
1	66	F	LAD	PTCA	14 days	-	+	-	-
2	51	M	LAD	Ath	27 days (58)	+	-	-	-
3	66	M	RCA	PTCA	56 days	-	-	-	-
4	67	M	LAD	PTCA	80 days	+	-	-	-
5	60	M	CX	PTCA	88 days	+	-	-	NA
6	75	M	LAD	PTCA	95 days	-	-	-	NA
7	56	M	LAD	PTCA	113 days	+	+	-	NA
8	52	M	LAD	PTCA	126 days (201)	+	-	-	NA
9	40	M	LAD	Ath	133 days	+	-	-	-
10	71	F	LAD	PTCA	134 days	+	+	-	NA
11	49	M	LAD	Laser	165 days	+	-	-	NA
12	64	M	LAD	Ath	195 days	+	-	-	NA
13	58	M	RCA	PTCA	597 days	+	-	-	NA

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Chapter 10

Donor origin of intimal smooth muscle cells in a de novo human coronary artery stenosis after heart transplantation: polymerase chain reaction analysis of VNTR loci

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(submitted)

Abstract

Smooth muscle cells are the predominant cell type found in the intimal lesions of atherosclerosis and restenosis following percutaneous transluminal coronary angioplasty. Animal studies have suggested the cells are derived from the vessel wall and not the circulation, although it has never been shown in man. In this study, tissue was removed by a Simpson coronary atherocath from a severe narrowing that had developed in the circumflex artery of a male who had undergone an orthotopic cardiac transplantation almost 5 years previously. The origin of the cells (donor versus recipient) was determined by amplifying the DNA by the polymerase chain reaction and comparing the electrophoresis patttern of three separate highly polymorphic variable number of tandem repeat (VNTR) gene loci in the atherectomy specimen, a donor myocardial biopsy performed immediately after the transplant, and a sample of the recipient blood. The DNA profiling results for the three VNTR loci showed that the coronary intimal tissue and the donor myocardial biopsy share the same alleles and that this pattern consistently differed from that of the recipient's blood. These results support the concept that the vascular intimal lesions typically seen in atherosclerosis and restenosis consist primarily of smooth muscle cells that are derived from within the vessel wall and not from the circulation.

key words: atherosclerosis, restenosis, smooth muscle cells, polymerase chain reaction

INTRODUCTION

Focal intimal thickening, common to primary atherosclerotic lesions and restenosis after mechanical coronary interventions such as percutaneous transluminal coronary angioplasty (PTCA), is a non-specific response of the vessel wall to a variety of injuries. The predominant cellular component in fibrous plaque is the smooth muscle cell (1). It remains unknown whether the smooth muscle cell in human coronary lesions is derived from cells circulating in the blood or from the vessel wall itself. Evidence in support of either possibility has appeared in the literature although animal studies have suggested that the tunica media or subintimal space is the source of the smooth muscle cells (2-5). Atherosclerotic lesions developing in the coronary arteries after orthotopic heart transplantation provide a unique opportunity to identify the origin of the cells constituting the atherosclerotic plaque since the donor and recipient invariably differ in their genetic content. Such genetic differences can be shown by electrophoretic analysis of alleles from the highly polymorphic Variable Number of Tandem Repeat (VNTR) gene loci which have a widespread occurrence in the human genome (6,7). The D1S80 (8), Apo B 3' VNTR locus (9) and the D17S5/D17S30 VNTR locus (10) represent such independent and highly polymorphic DNA markers which can be used for biological identification purposes. In this case report, we show by electrophoretic analysis of alleles from three separate VNTR loci amplified by the polymerase chain reaction (PCR) technique (11), that the smooth muscle cells present in a de novo human coronary lesion after heart transplantation are derived from the donor vessel wall and not from circulating recipient cells.

Case History: A 37 year old male underwent orthotopic cardiac transplantation in January 1985 because of endstage biventricular heart failure due to idiopathic dilated cardiomyopathy. The donor heart came from a 34 year old woman with no previous cardiac history. At the conclusion of the transplantation, a baseline myocardial biopsy (B1) was taken from the right ventricle and immediately frozen in isopentane in liquid nitrogen at -70° C. As part of routine follow-up, yearly coronary angiograms were performed (Figure 1A). In January 1990, a new 60% diameter stenosis had developed in the mid circumflex coronary artery (Figure 1B). On the basis of a reversible Th^{201} perfusion defect in the left ventricular posterior wall, the patient underwent percutaneous directional coronary atherectomy in October 1990 without complication (B2). Venous blood (Blo) was drawn from the patient for DNA profiling studies. The tissue removed from the coronary artery was immediately frozen in isopentane in liquid nitrogen at -70° and representative sections were stained with haematoxylin-azofloxine and van Gieson stains. The remainder of the coronary tissue, the myocardial biopsy and the donor blood were then sent for PCR analysis of the three VNTR loci.

Figure 1a. *Angiogram from 1989. No discrete lesions are evident.*

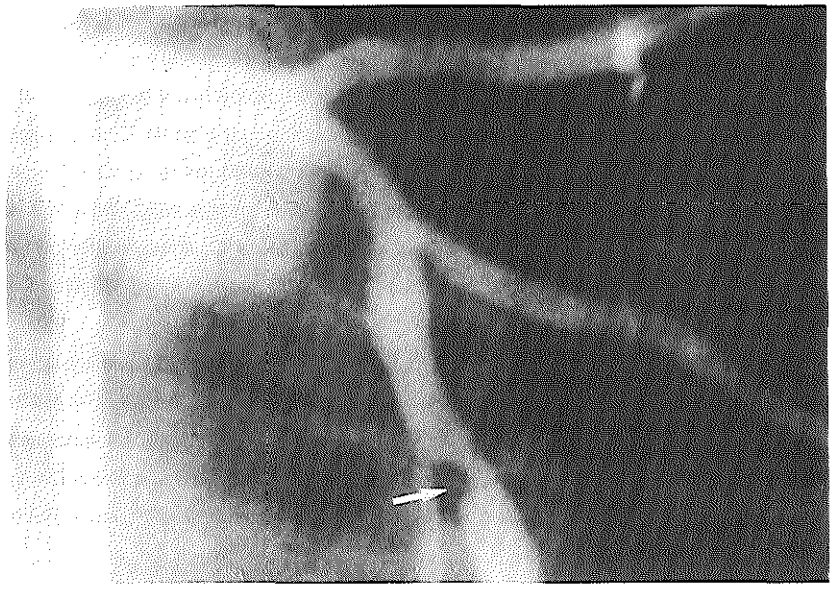

Figure 1b. *Angiogram from 1990. A 60% eccentric stenosis has developed in the circumflex artery (arrow).*

MATERIALS AND METHODS

Genomic DNA was isolated from the biopsies (± 1 mm^3) by lysing the tissue in a solution of 400 µl containing 10 mM Tris-HCl, pH 8.0; 10 mM Na_2EDTA; 100 mM NaCl; 0.5% SDS; 500 µl proteinase K (Merck) and incubating at 65°C for 4 h under gentle shaking. The lysis mixture was extracted once with Tris-saturated phenol and once with chloroform-isoamylalcohol (24:1) and subsequently ethanol-precipitated. The DNA was dissolved in a solution containing 10 mM Tris-HCl (pH 8.0) and 1 mM Na_2EDTA. Biopsy #1 was dissolved in 15 µl (800 ng/µl) and Biopsy #2 was dissolved in 20 µl (20 ng/µl). For DNA isolation from blood (Bl), the erythrocytes were first lysed by adding 3 volumes of lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$ and 1 mM Na_2EDTA) and incubating on ice for 30 min. After centrifugation, the pellet containing the white blood cells, was resuspended and incubated in 10 mM Tris-HCl (pH 8.0); 10 mM Na_2EDTA; 100 mM NaCl; 0.5% SDS; 500 µg/ml proteinase K (Merck) at 65°C for 4 h under gentle shaking. The lysis mixture was extracted once with Tris-saturated phenol and once with chloroform-isoamylalcohol (24:1) and subsequently ethanol-precipitated. The DNA was dissolved in 10 mM Tris-HCl (pH 8.0) and 1 mM Na_2EDTA to a concentration of approximately 800 ng/µl. The yield of genomic DNA from whole blood was 35 µg/ml blood.

For the polymerase chain reaction (PCR) analysis, 10 ng of genomic DNA was added to a solution (with a final volume of 50 µl) containing 1.5 mM $MgCl_2$ (D1S80 and Apo B 3' VNTR) or 1.0 mM $MgCl_2$ (D17S5/D17S30), 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 µM dNTPs (Pharmacia, Sweden), 10 % glycerol (Merck, Germany), 0.2 mg/ml acetylated BSA (Gibco-BRL, USA), 300 ng primers, 1 unit Taq polymerase and 5% of a detergent solution according to the manufacturers (Gibco-BRL, USA) instruction. Before entering the temperature cycling, the solutions were overlaid with 50 µl Nujol mineral oil (Perkin-Elmer, USA). After an initial denaturation of 4' at 94°C, 30 cycles of incubations at different temperatures specific for each locus (the D1S80 locus: denaturation for 2' at 94°C, annealing for 2' at 65°C, and primer extension for 4' at 72°C with a final primer extension for 10' at 72°C; the Apo B 3' VNTR locus: denaturation for 4' at 94°C, annealing/primer extension for 6' at 58°C and a final extension for 10' at 58°C; the D17S5/D17S30 locus: denaturation for 4' at 94°C, annealing for 30" at 55°C, primer extension for 4' at 72°C and a final extension for 10' at 72°C) were performed in a thermal cycler apparatus (BIOMED, Germany).

After completion of the PCR reaction, samples of 5-30 µl of the solution were analysed on a 9 % polyacrylamide gel (acrylamide:bisacrylamide=37:1) run in 1xTAE (40 mM Tris-HCl, pH 7.4; 20 mM Na-acetate, 1 mM Na_2EDTA) for 1.5 hours at 200 V in a vertical slab gel electrophoresis apparatus placed in a buffertank kept at 50°C (12). After electrophoresis the separation pattern was stained in a solution containing 30 µg/ml ethidium bromide and documented by polaroid photography under 302 nm (UVP products, USA).

RESULTS

The specimen contained cells with the morphologic appearance of smooth muscle cells but were not arranged with the architecture of a tunica media (Figure 2). The smooth muscle cell identity was confirmed through immunoreactivity for smooth muscle cell specific alpha actin as detected by immunohistochemistry with monoclonal antibodies (Sigma, St. Louis) (Figure 3). A few cells stained with leukocyte common antigen. Monoclonal antibodies directed to macrophages and endothelial cells were negative.

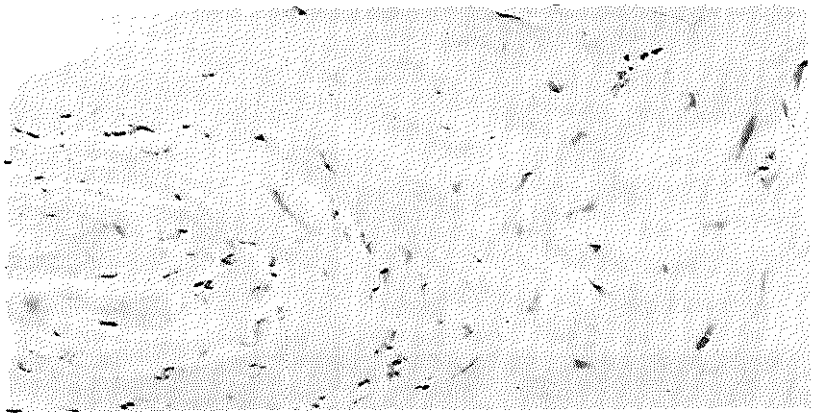

Figure 2. *Coronary atherectomy specimen (haematoxylin-azofluoxine stain) containing intimal tissue.*

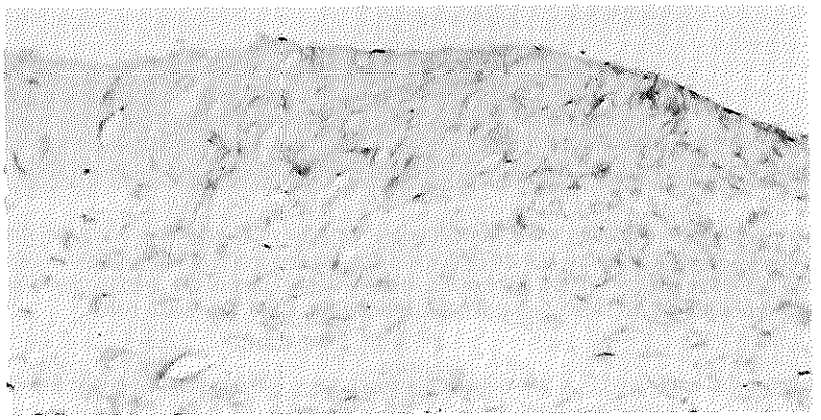

Figure 3. *Coronary atherectomy specimen. Smooth muscle cells are identified by the dark staining. An antibody specific for smooth muscle alpha actin has been coupled to a peroxidase reaction and is responsible for the dark colour.*

The results of gel electrophoretic separation obtained after PCR amplifcations of alleles of the D17S5/S30 locus (lanes 2-4), the D1S80 locus (lanes 6-8), and the Apo B 3' VNTR locus (lanes 11-13) are shown in Figure 4. The donor myocardial biopsy (B1) and the coronary intimal tissue (B2) shared the same alleles and this pattern consistently differed from that of the recipient's blood (Blo). For the D17S5/S30 locus (lanes 2-4) PCR products of 372 base pairs (bp) and 550 bp (the latter one is faintly visible due to inefficient amplification of larger alleles of this locus; see Horn et al., 1989) were observed in the blood. In B1 and B2, only the 372 bp was faintly visible, and in addition, these biopsies shared a fragment of 236 bp, which would correspond to homozygosity of the locus for this allele in both biopsies. Similarly, for the D1S80 locus (lanes 6-8), two alleles, of 420 bp and 460 bp, were observed for the blood DNA while the biopsies were homozygous for an extra band of 510 bp.

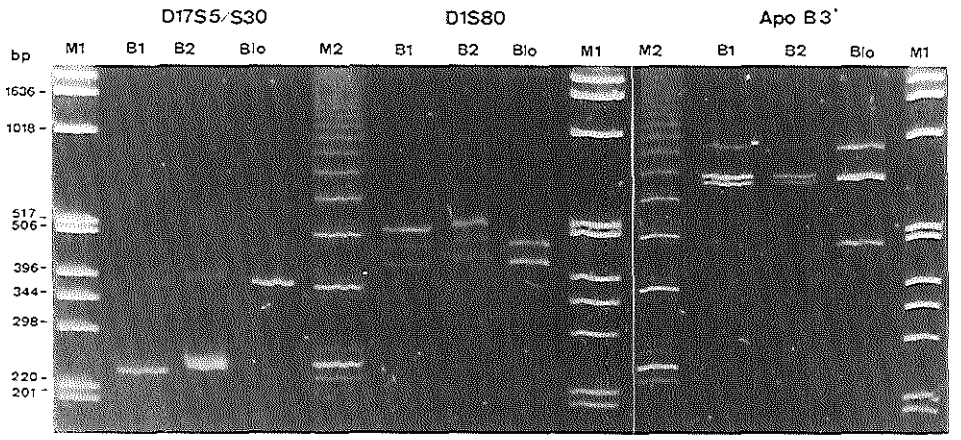

Figure 4. *Gel electrophoretic analysis of products obtained after PCR amplification of the three VNTR loci D17S5/S30, D1S80, and Apo B 3' from genomic DNA isolated from donor myocardial tissue (B1), coronary intimal tissue (B2) and blood (Blo) of the recipient. M1 is part of the "1 kb ladder" size marker and M2 is part of the "123 bp ladder" size marker (Gibco BRL, USA). bp=base pairs.*

PCR amplification of the Apo B 3' VNTR locus (lanes 11-13) in genomic DNA isolated from recipient blood (biopsy 3) resulted in the detection of three fragments of 570, 720 and 880 base pairs. The generation of three bands, which all are within the expected size range of the Apo B 3' VNTR alleles, instead of the expected two fragments is unexplained. Nevertheless, both in B1 and B2, an extra band

of 690 bp was observed. Because two bands (690 and 720 bp) in the biopsies are more intense than the other bands, the biopsies are heterozygous at this locus with alleles of 690 and 720 bp. Apparently, one of these alleles (the 720 bp) is shared with the blood. The slight upward bandshift seen in all lanes containing PCR products from biopsy #2 (most notably in lanes 3 and 7) is most likely caused by the larger sample volume, and consequently a higher absolute amount of salt was loaded on the gel (30 µl for biopsy #2 compared to 5 µl for biopsy #1).

Faint bands corresponding to the blood pattern (Blo) were evident for all three gene loci (except the 570 bp allele of the Apo B3' gene) in biopsies 1 and 2. The relative intensity differences of blood-specific bands and biopsy-specific bands for the three loci, as measured by image analysis of the polaroid photo, suggest a 10% contamination of blood in biopsies #1 and #2. This is most likely due to unavoidable contact of the recipient's blood with both the myocardial biopsy (taken immediately after completion of the heart transplantation procedure) and the atherectomy obtained coronary specimen.

DISCUSSION

In a previous review on the pathogenesis of atherosclerosis, Ross wrote that "It may never be possible to determine with certainty the specific events that lead to atherosclerosis in humans" (1). As a result, many concepts of the sequence of events have been based on animal studies. Recent advances in molecular biology combined with recent technological innovations such as the atherectomy catheter have allowed us to biopsy and study coronary material to address basic questions related to the development of an atherosclerotic lesion. Coronary artery lesions in a transplanted heart are likely the only human clinical setting in which one can define the origin (blood borne versus vessel wall) of the smooth muscle cells found in the intimal lesion.

In this case, an advanced coronary lesion consisted primarily of donor cells that originated from within the vessel wall. A slight degree of DNA from the recipient was seen in the atherectomy sample as well. There are two possible explanations. Since a comparable band was seen in the myocardial biopsy (taken directly after transplantation) as in the atherectomy specimen, contamination with the recipient's blood during removal of the specimens is probably the main reason. However it is also possible that a small contribution to the DNA band is due to the small number of lymphocytes and posssible macrophages that were part of the lesion. The DNA profiling results for the three VNTR loci analyzed support the hypothesis that the myocardial and coronary intimal biopsies are derived from the same individual and are different from the pattern seen in the recipient's blood. Immunohistochemistry studies of the coronary intimal tissue confirm that the smooth muscle cells are the main cell type found in the lesion and must therefore originate from within the vessel wall, although it can not be stated whether the cells are from the media or the subintimal space. Although it may be argued that the coronary lesion predated the heart transplantation, the results of the yearly angiograms prior to 1990 along with the fact that the heart donor was a young woman without a cardiac history are convincing evidence that the lesion developed

after cardiac transplantation.

In conclusion, the results of this study support the concept previously suggested by animal studies that the smooth muscle cells in vascular intimal lesions typically seen in atherosclerosis and restenosis are derived from within the vessel wall and not from the circulation.

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Chapter 11

Human coronary smooth muscle cells in culture: Phenotypic features, proliferation and extracellular matrix production

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(in preparation)

Abstract

Cell cultures were attempted in coronary tissue that was obtained from 85 atherectomy procedures (82 patients) using the Simpson coronary Atherocath®. Primary de novo lesions and restenosis after a previous coronary intervention accounted for 78% and 22% of the procedures, respectively. Cell outgrowth was seen in 40% of cultures although serial (>2) passages were obtained in only 10 patients. Cell growth was enhanced by the explant technique and in tissue with cell density >700 cell/ mm^2. No differences were evident between the type of lesion and the ability to serially passage cells.

Two types of cells were observed in culture. The predominant type had the typical morphological features of smooth muscle cells. These cells were of the synthetic phenotype based on the low incidence of cells that stained positively with antibodies to smooth muscle cell alpha-actin and the appearance of abundant Golgi apparatus and endoplasmic reticulum and peripheral location of myofibrils on electron microscopy. In a few primary cultues, cells that resembled macrophages proliferated for up to 2 weeks and could not be passaged.

In-vitro studies of proliferation rates and matrix production were performed in the coronary cells in passages 2-5 and compared with smooth muscle cells isolated from the media of human umbilical arteries. Significantly slower population doubling times were seen in the coronary cells compared with the control umbilical cells (51 ± 9 hours versus 33 ± 12 hours, $p < 0.05$). However, matrix synthesis was significantly greater in the coronary cells than the umbilical cells. Collagen production was 50% higher and glycosaminoglycan synthesis was more than two- fold greater in the coronary cells.

Smooth muscle cells from primary atherosclerotic and restenotic coronary lesions can be successfully grown in culture. The specialized functions of proliferation and matrix production that are responsible for the development of these coronary artery lesions can be studied in this model. Preliminary data suggests that the cells originating from coronary plaques are particularly active in matrix synthesis.

INTRODUCTION

The pathologic processes in both coronary atherosclerosis and restenosis following percutaneous transluminal coronary angioplasty (PTCA) are characterized by the proliferation of intimal smooth muscle cells (SMC) and extracellular matrix formation (1,2). Cell culture is an important aspect of research in this field since specific properties of individual cell types can be studied without the complexities of interactions occurring in the intact organism. In-vitro studies of the proliferative and synthetic properties of SMCs have been primarily performed in cells isolated from normal media of animal vessels or from experimentally induced intimal thickenings (3). The study of SMCs from human atherosclerotic lesions has been limited and predominantly restricted to peripheral arteries that have been surgically amputated or removed post-mortem (4,5).

The development of a percutaneous directional atherectomy catheter that can remove obstuctive material from the vascular wall has opened new possibilities of vascular research. It is now possible to study human vascular tissue from a much broader range of patient than was previously possible. In particular, we now have access to material from restenosing lesions that in the past has been extremely difficult to obtain. Initial work in culturing material removed by the atherectomy device was performed in tissues obtained from peripheral vessels (6-9).

In September 1989, coronary atherectomy was initiated at the Thoraxcenter. With this unique opportunity of acquiring human coronary tissue from a variety of patients and clinical settings, a cell culture programme was set up to isolate SMCs and to define the important baseline properties of these cells. We have focussed on the aspects of these cells that are important in the development of vascular intimal lesions, namely growth characteristics and synthetic capacity. Collagen and proteoglycans are important components of the extracellular matrix produced by the SMC and are thus central to studies of the synthetic behaviour of SMCs. Collagen is a major structural element of the extracellular matrix of the arterial wall, comprising 20-50% of the dry weight (10,11). Proteoglycans are macromolecules that consist of negatively charged complex carbohydrates (sulfated glycosaminoglycans) linked covalently to the serine residues of a central protein core. Glycosaminoglycans are made up of repeating disaccharides, each containing a uronic acid linked to a hexosamine (amino sugar) residue. In the vessel wall, proteoglycans are usually a minor component, 2-5% of dry weight. However, proteoglycans accumulate in atherosclerotic lesions and in the intimal hyperplasia that occurs after vessel injury (12,13). Proteoglycans have important roles in cell adhesion and migration, cell proliferation, lipid metabolism and thrombosis (14) and may also be important in the progression of lesions since they are involved with the binding of macromolecules in the intima, including the uptake of LDL by macrophages.

In this chapter, we report the preliminary results of studies of the proliferative behaviour and matrix production of SMCs isolated from coronary artery lesions compared to SMCs originating from the normal media of human umbilical arteries

METHODS

Patients with suitable coronary anatomy underwent percutaneous coronary atherectomy following informed consent and according to a protocol approved by our Institutional Review Board. Patients were specifically classified according to the clinical status and the type of lesion (primary atherosclerotic plaque or restenosis following previous angioplasty). The atherectomy procedure using the Simpson coronary atherocath^{R} has been described elsewhere (15). Under sterile conditions, the specimens were removed from the housing of the atherectome, washed with 0.9% saline and placed in M199 culture medium without serum supplementation for transfer to the culture laboratory.

Histology

The specimens were cut with a sterile knife to a size of about 1 mm by 1 mm. One to two pieces were immediately fixed in 10% buffered formalin, and in some cases in a solution of glutaraldehyde-formaldehyde (4CF-1G) for transmission electron microscopy. The maximal cell density (in the initial 12 patients that had the explant method) of the tissue specimens removed by atherectomy was determined as follows: In haematoxylin and azofloxine stained cross-sections, the number of cell nuclei was assessed in several fields by a computerized morphometry system (IBAS, Kontron, Oberkochen, Germany). The maximum value recorded was used for the determination of cell density which was expressed as cell number/ mm^2 intimal tissue.

Cell Culture

The remaining tissue was placed in cell culture according to the two methods described by Bauriedel et al (6-9). First, tissue explants were placed on fibronectin coated (10 ug/cm^2) glass cover slips in 2 cm^2 wells and then the culture medium (M199, supplemented with glutamine, 10% human serum, 10% fetal calf serum, penicillin 100 IU/ml and streptomycin 0.1 mg/ml) was added. In primary cultures, conditioned medium from actively growing cell lines was mixed 1:1 with the culture medium described above. For secondary cultures, culture medium was not supplemented with conditioned medium. Second, the tissue was enzymatically disaggregated for 30 minutes at 37°C in dispase grade II (2.4U/ml, Boehringer Mannheim) containing 1 mg/ml collagenase Worthington CLS III (229 U/mg, Worthington Biochemical Corporation, New Jersey) and then the solution was supplemented with 20% human serum and centrifuged. The supernatant was discarded and the pellet was again incubated in the following enzyme mixture: 10 ml of HEPES-buffered culture medium containing 18 mg collagenase, 2 mg elastase from pancreas (Boehringer Mannheim, Germany) and 10 mg trypsin inhibitor from soybean (Sigma) for 60-180 minutes at 37°C to isolate individual cells which were then plated as previously described for the explants. Cell culture was carried out in a 5% CO_2 incubator at 37°C. At confluency, cells were subcultured by trypsin.

SMCs obtained from the media of human umbilical arteries were used as control cells.

Immunological Identification

Cells of passage 2-4 were seeded on round glass cover slips at a density of 5000 cells/mm^2. After 48 hours, the cover slips were rinsed in PBS (pH 7.4) and then fixed in acetone at -20°C. Immunostaining was performed with monoclonal antibodies directed against SMC alpha-actin (Sigma, St. Louis, USA) using an indirect conjugated peroxidase procedure. Specificity of staining was done by exclusion of the primary antibody. In addition to coronary and umbilical artery SMCs, cultured human skin fibroblasts were included in this experiment in order to provide a negative control.

Electron Microscopy

For electron microscopy, the cells cultured in 35 mm diameter petridishes (Costar,) were fixed in a phosphate buffered mixture of 4% paraformaldehyde and 1% glutaraldehyde, postfixed in 1% OsO_4, dehydrated in a graded series of alcohol and finally embedded in epon. For the embedding in epon, embedding-capsules filled with epon were placed upside down on the cell monolayer. After polymerisation of the epon, the epon blocks were separated from the plastic by immersion in liquid nitrogen. Ultrathin sections were cut on Reichert OmLI3 Ultramicrotome (Reichert, Wien, Austria), stained with uranyl acetate and lead citrate and examined in a Philips E.M. 400 electron microscope.

Population Doubling Times

Cells in the second to fifth passage were seeded in 2 cm^2 culture wells at a density of 3000 cells/cm^2. At 24 hours after seeding and on every third day thereafter, the medium was exchanged. At appropriate times, cells of three wells were washed with PBS, trypsinized for 5 minutes at 37°C, and were counted in a haematocytometer. The mean values for cell number at each counting interval were plotted on logarithmic scale versus the number of hours in culture and population doubling times were derived.

^{3}H-Thymidine Incorporation Assay

Cells were grown on coverslips in 2 cm^2 well plates. During the logarithmic phase of cell growth (day 5-7), ^{3}H-thymidine stock solution was added to the medium (final concentration 0.1 uCi/ml) for 24 hours. The experimental medium was then removed and the monolayer was rinsed with PBS. The cover slips were fixed in Bouin fixative, coated with photographic emulsion Ilford K_2 and developed after exposure for 10 days. Nuclei were stained with haemotoxylin and eosin and the thymidine index was determined as the proportion of nuclei showing silver grains in the light microscope. At least 200 cells were counted on each triplicate slide.

Determination of collagen synthesis

Collagen was determined with a bacterial-collagenase digestion method modified from Peterkofsky and Degelmann (16). In confluent 2 cm^2 culture wells, cells were incubated for 48 hours at 37°C in 0.5

ml of M199 supplemented with 5% human serum, 5% fetal calf serum, 2 uM ascorbic acid, and 5 ul H^3-proline (10uCi/ml). Preliminary experiments from Janssen Research Foundation in Beers, Belgium indicated that it was unnecessary to separately determine the cell associated collagen and the collagen in the medium. All experiments were performed in triplicate.

At the completion of the incubation period,the medium was removed from the monolayer and both were separately precipitated as follows: the medium was precipitated by 20% TCA 1 mM proline at 4°C for 24 hours. After centrifugation (5000g x 10 minutes at 4°C), the pellet was washed with 20% TCA 1 mM proline and then with 10% TCA 1mM proline. The monolayer was precipitated with 20% TCA 1mM proline overnight, then washed with 20% TCA 1 mM proline and then 19% TCA 1 mM proline. The precipitate was dissolved in 0.5 ml 0.2N NaOH overnight at 4° in a humidity chamber and 25 ul was counted in Instagel II. Then 400 ul from the dissolved monolayer was added to the pellet derived from the medium and 50 ul was counted in Instagel II. The rest of the resuspended monolayer (75 ul) was used to determine the total amount of protein with the Pierce BCA protein assay (Pierce Rochford, Illinois, USA) utilizing BSA (bovine serum albumin) as standard (17).

Two 75 ul samples of the combined medium and monolayer were mixed with 100 ul of a HCl buffer (75 ul 1M HEPES pH 7.3, 2.5 mM $CaCl^2$ with 120 ul 0.15N HCl). The samples were then incubated with a collagenase solution (7 ul *clostridium histolyticum* -Type III, Calbiochem 2600 U/ml to 25 ul [TRIS-HCl (50mM) pH 7.6 + 5 mM $CaCl_2$]) or control (minus the collagenase) for 2 hours at 37°C and precipitated in ice-cold 10%TCA/ 5% Tannic acid, and centrifuged. The supernatant was collected and 500 ul was counted in Instagel II. Collagen synthesis was expressed as nmoles [^{3}H]-proline/ug total cell protein /hour.

Determination of Sulfated Glycosaminoglycan Synthesis

In confluent 2 cm^2 culture wells, cells were incubated for 48 hours at 37°C in 0.5 ml of M199 supplemented with 5% human serum and 5% fetal calf serum and 20 uCi/ml ^{35}S-sulfate (10uCi/ml). The medium from ^{35}S-sulfate labelled cells was washed with PBS to a final volume of 2.5 ml. Two hundred and fifty microliters were applied onto a disposable Sephadex G-25M (PD10, Pharmacia) gel filtration column equilibrated and run in urea 8M/Triton X100 0.5%/NaCL 0.15M/Na acetate 50 mM pH 6.0 (18). Fractions of 300 ul were collected, mixed with Instagel II and fractions 8-45 were counted. The sum of the counts in the initial peak (dpm) was used to determine the degree of glycosaminoglycans synthesis which was expressed as nmol/ug protein.

Statistical Analysis

Comparisons between coronary cells and the control umbilical cells were performed using the two-sample t test. A p value < 0.05 was considered statistically significant.

RESULTS

Culture Success Rates

Cell cultures were attempted in coronary tissue obtained from 85 atherectomy procedures (82 patients) in a 2 year period (September 1989 to September 1991). Twenty (22%) of the procedures were performed for restenosis. Cell outgrowth, was seen in 40% of cultures although serial passages (up to 7) were obtained in only 10 patients (12%), all by the explant method (Table 1). Cells started to grow out from the explants after 4-8 days and confluent multilayer primary cultures in the 10 patients were established in 4-6 weeks. Cell growth was enhanced by the explant technique, and in tissue with cell density > 700 cells/mm^2 (Figure 1). The enzyme technique was abandoned after the initial 9 patients in favour of the more successful explant method. No differences were evident between the ability to serially passage cells and the type of lesion (primary atherosclerotic or restenosis).

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1) METHOD OF CULTURE

	PRIMARY	RESTENOSIS	TOTAL
ENZYME	1/5	1/4	2/9 (22%)
EXPLANT	23/59	9/18	32/77 (42%)

2) CELL DENSITY/ mm^2 TISSUE (12 PTS)

UNSUCCESSFUL 231 ± 200 (RANGE 35-500)
SUCCESSFUL 922 ± 215 (RANGE 687-1320)

Figure 1. *The two determinants of success in establishing cell outgrowth in cultures were explant nethod (cell outgrowth in 40% of lesions attempted) and high cell density in tissue that was removed (> 700 cells/ mm^2 tissue). Primary= primary atherosclerotic lesion.*

Cell types

Two types of cells were seen in culture. The predominant type was seen in subconfluent cultures as a network of multilayered elongated cells in interlacing bands separated by empty areas. In confluent cultures the cells formed whorls giving a hill-and-valley pattern typical of cultured SMCs (Figure 2a). In all serially passaged cell lines, this pattern was seen and persisted throughout the passages.

In a small number of primary cultures, a second type of cell was seen that was rounded in appearance and usually contained small inclusions. These cells would proliferate for up to 1-2 weeks

and then stop (Figure 2b). The appearance and size of these cells was typical of macrophages (immunohistochemistry studies pending).

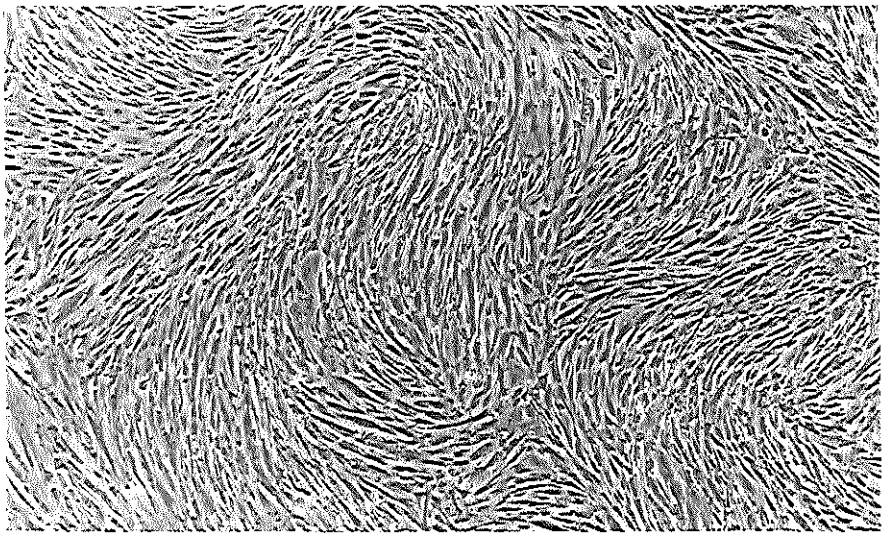

Figure 2a. *Phase contrast micrograph showing hill-and-valley pattern typical of smooth muscle cells in culture.*

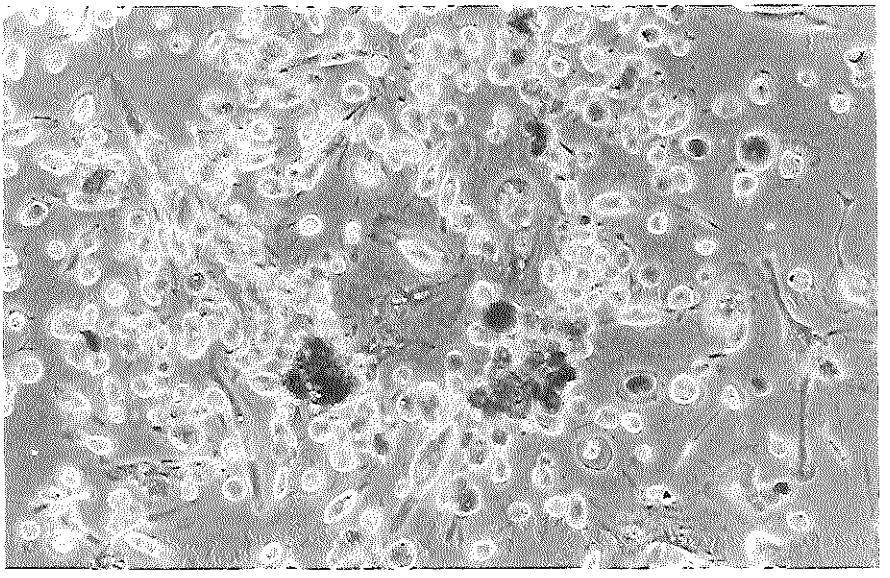

Figure 2b. *Phase contrast micrograph showing round cells containing inclusions.*

Transmission Electron Microscopy

The cells in culture had the phenotypic features of a myofibroblast (synthetic SMCs). The cytoplasm contained numerous endoplasmic reticulum, Golgi apparatus and ribosomes (Figure 3a). Myofibrils were present in the periphery of some of the cells (Figure 3b).

Immunohistochemistry

The human skin fibroblasts (negative control) demonstrated non-specific background staining (Fig. 4a). A minority of the coronary artery cells (approximately 10%) in secondary cultures contained fibers that stained with anti-SMC alpha-actin characterizing these cells as SMCs (Figure 4b). These cells were larger and well-spread on the culture plate. The majority of the cells had diffuse background staining with alpha-actin without obvious fiber formation and were more elongated in shape. Variable staining with antibodies to SMC-specific alpha-actin was evident in the control umbilical artery cells (Fig. 4c). In most of the cell strains the majority of the cells contained alpha-actin positive fibers (even up to the seventh passage), but in a few cell strains the situation was similar to the coronary cells in culture. The umbilical artery cells also had the hill and valley culture morphology.

Population Doubling Times (Table 2)

The coronary cells had a 50% longer doubling time than the control umbilical SMCs (51 ± 9 hours versus 33 ± 12 hours, $p < 0.05$). No significant differences were found between coronary cells from primary atherosclerotic and restenosing lesions.

Thymidine Labelling (Table 3)

Both umbilical and coronary artery cells had a high percentage of cells proliferating during the 24 hour period of labelling (Fig. 5a,b) although the former cells had a larger proportion of proliferating cells (71% versus 47.5%).

^{3}H-Proline Incorporation (Table 4)

Collagen synthesis, based on the incorporation of radioactive proline, was not significantly greater in coronary SMCs (0.0335 ± .0124 nmoles proline/ ug total cell protein) than control umbilical SMCs (0.0188 ± 0.0062). No significant differences were found between primary atherosclerotic and restenosis lesions. In the coronary cells, there was a trend towards decreased collagen synthesis with later passages.

^{35}S-Sulfate Incorporation (Table 5)

Glycosaminoglycan synthesis, based on the incorporation of radioactive sulfate, was 2.2-2.4 times greater in coronary cells than in the control umbilical SMCs.

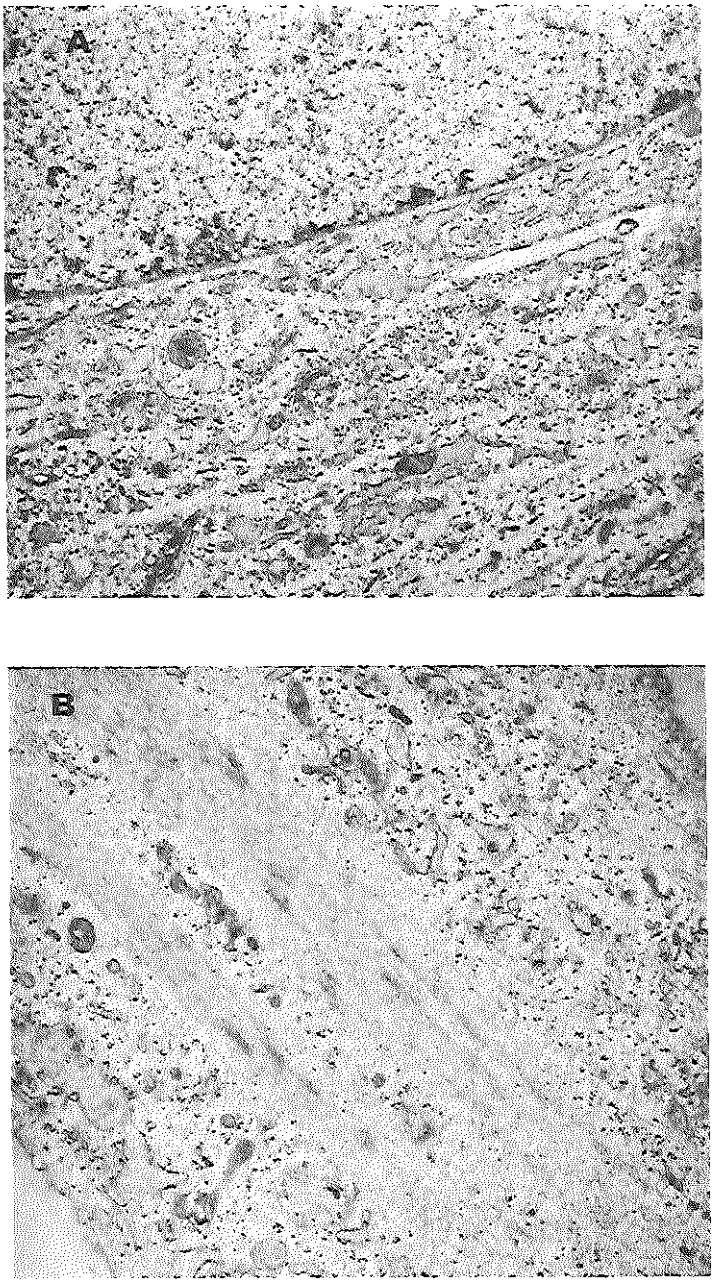

Figure 3. *Transmission electron micrographs showing synthetic phenotype of smooth muscle cells in culture. A) Abundant cytoplasmic organelles including Golgi apparatus, endoplasmic reticulum and ribosomes. B) Myofibrils were located in the periphery of some cells.*

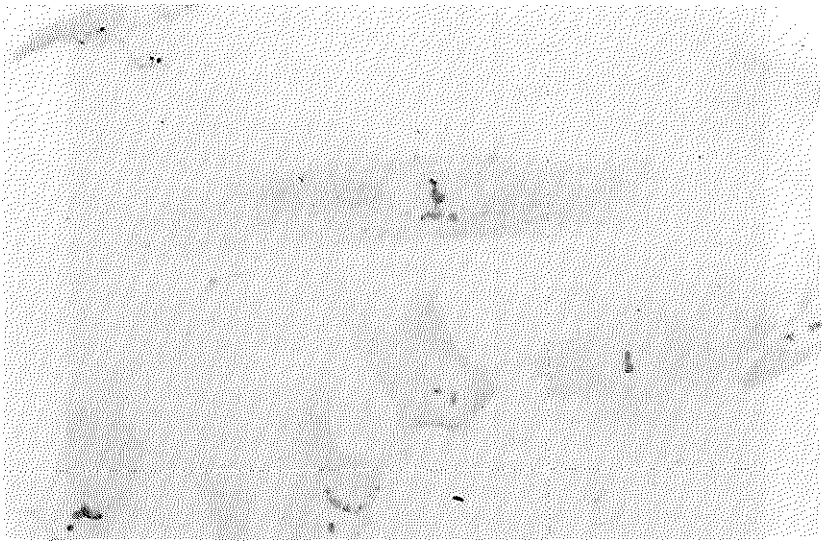

Figure 4a. *Monoclonal antibody directed against smooth muscle cell specific actin (1/400 dilution). fibroblast: negative staining, only nonspecific background staining.*

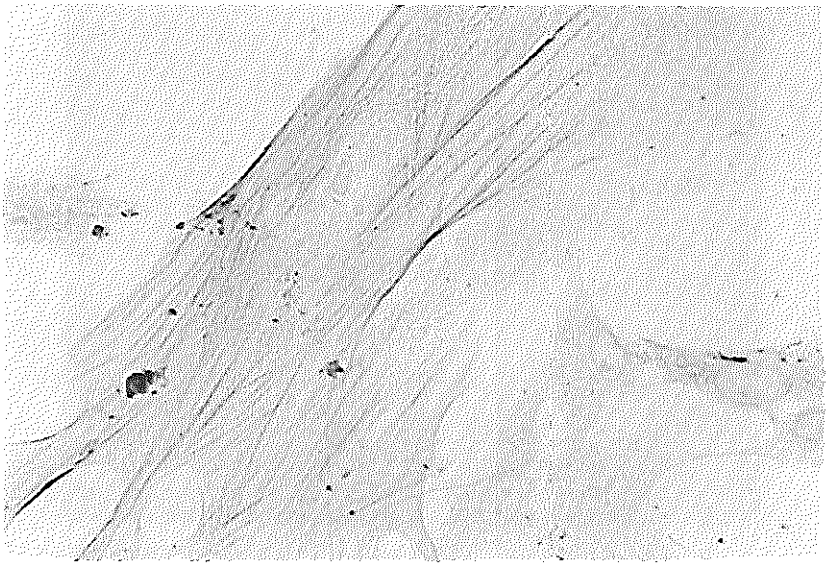

Figure 4b. *Coronary artery cells: a few cells contain fibers that stain positive for alpha-actin but the majority of cells only show nonspecific background staining.*

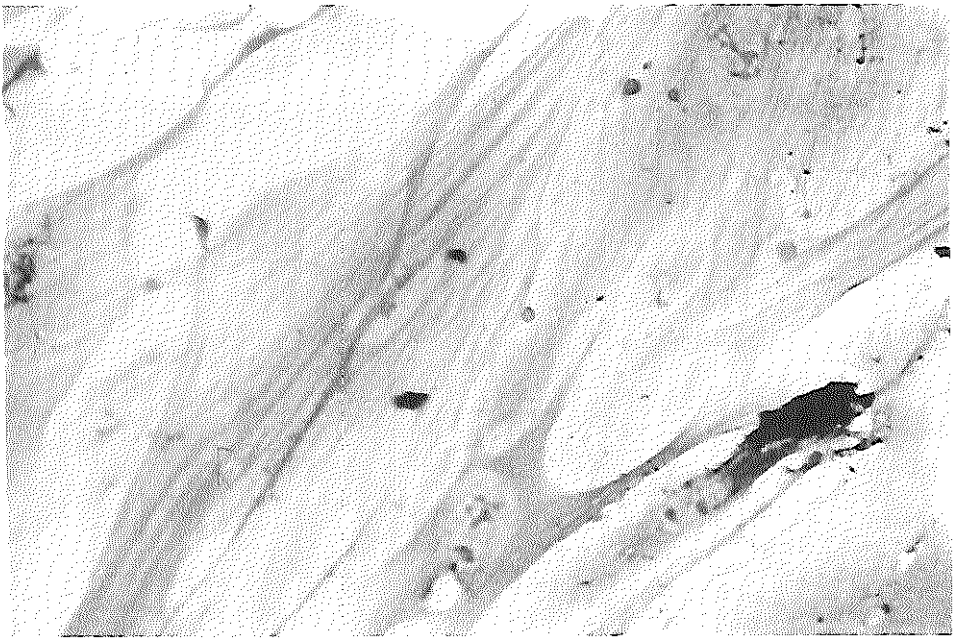

Figure 4c. *Umbilical artery cells: some fibers stain positive for alpha-actin.*

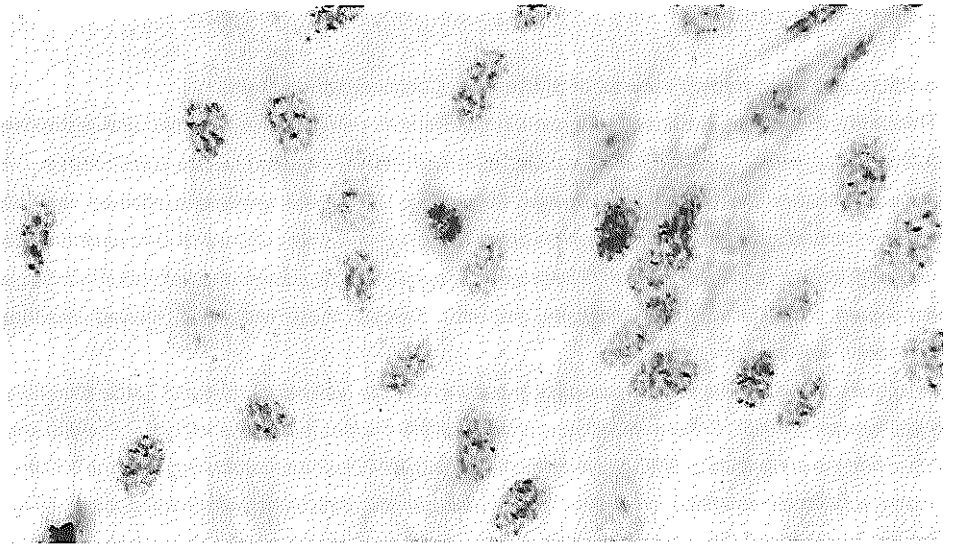

Figure 5a. *Thymidine labelling of proliferating cells. Control umbilical cells: the majority of cells (71%) are labelled (multiple dark grains in the nucleus)*

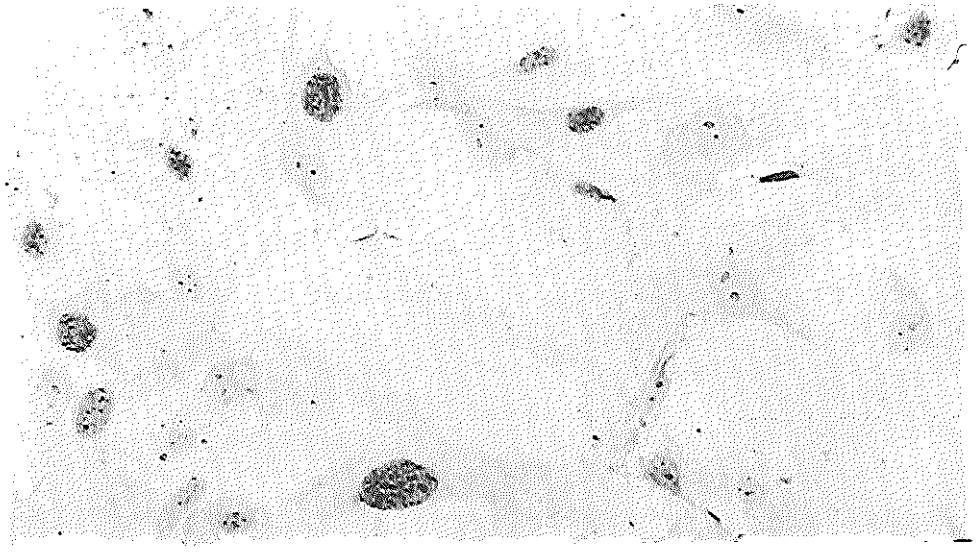

Figure 5b. *Thymidine labelling of proliferating cells. Coronary artery cells- staining is present but less marked than in the umbilical cells.*

DISCUSSION

Feasibility and Phenotypic Features of SMCs from Coronary Atherectomy

This study shows that SMCs can be grown in culture from primary atherosclerotic and restenosed human coronary artery lesions. Tissue characteristics (high cell density) and culture conditions (explant technique) enhance the overall success rate. Since only 12% of the specimens attained extended passage in culture, considerable selection bias may be occurring within culture and care must be taken in extrapolating the results to the overall population. It remains to be shown whether culture yields can be improved by other methods (collagen gels etc). Despite these limitations, several important observations emerge from this study.

First, the coronary SMCs in early passages in culture have the features of synthetic SMCs (fibroblast-like appearance) (19-20). Consistent with this phenotype are the fact that only a minority of cells stained positive with the antibodies to SMC alpha-actin, the hill and valley culture morphology, and the presence of many subcellular synthetic organelles (rough endoplasmic reticulum and Golgi apparatus) and peripheral myofibrils on electron micrographs. Gabbiani et al have shown that the phenotypic modulation of the SMC from the "contractile" phenotype, typical of the medial SMC, to the "synthetic" phenotype found in embryogenesis, atherosclerotic lesions, and in experimental models of intimal thickening is

characterized by a shift in the cytoskeleton proteins from SMC-specific alpha-actin to beta- actin predominance (21-23). Studies in vascular SMCs in culture have previously demonstrated that cultured SMCs undergo differential expression of isoactins in relation to their growth state. Owens et al showed in rat aortic SMCs that SMC alpha-isoactin synthesis and content are low in subconfluent log phase growth cells but increase three-fold in density-arrested postconfluent cells (24). Similarly, Fager et al found an inverse relationship between the expression of contractile proteins (SMC specific alpha-actin) in stress fibers and the proliferative state of the cells (25). In growth arrested cells, 80% of cells showed stress fibers containing SMC-specific alpha-actin. However, only 4% of cells had positive staining with a monoclonal antibody against SMC alpha-actin after 4 days of growth in serum enriched growth medium.

It is uncertain from our study whether the coronary SMCs grown in culture underwent phenotypic changes in culture or already had a synthetic phenotype in the tissue prior to culture. The coronary cells in this study originated from advanced atherosclerotic and restenotic coronary intimal lesions. Although previous work has suggested that human atheromatous lesions have a low SMC alpha-actin content (22), we have seen extensive staining with monoclonal antibodies directed against SMC alpha-actin in atherectomy obtained tissue from 15 restenosis lesions (26). EM studies in three restenosis patients 83-135 days after stent placement showed that the majority (70-75%) of intimal cells had phenotypically contractile features (extensive myofibrils, few endoplasmic reticulum or Golgi apparatus). Therefore the synthetic cells in culture may be derived from the smaller population of synthetic intimal cells or from phenotypic changes induced by the culture conditions.

It must also be recognized that SMCs are not the only cells that could be grown in culture. The presence of proliferating macrophages was noted in 5 cultures although growth in our experimental conditions could not be maintained beyond 1-2 weeks.

Proliferation Rates

Significantly lower growth rates (50%) were seen in the coronary cells compared with the control umbilical medial SMC's. Furthermore, sustained growth to confluency in primary culture could not be achieved in 88% of the specimens. A differential proliferative response between intimal and medial SMCs in human peripheral and aortic vascular tissue has been previously described (5). Ross et al have postulated that the reduced proliferative capacity of SMCs derived from the intima suggest senescent changes due to multiple previous divisions in vivo. Dartch et al previously reported in a similar cell culture model that cultured SMC from restenosing lesions grew at a significantly higher rate than cells from primary atherosclerotic lesions (7). They also described features of senescence in cells grown from primary atherosclerotic lesions such as inability to subculture more than two times, extensive cellular debris, and a prominent microfilament network with cytoplasmic vacuoles. In the limited number of cell lines studied in our study, we did not observe these differences in culture between the two types of lesions. This may be related to the artery (coronary versus peripheral vessels in Dartsch study), age of

the restenosing lesions prior to the atherectomy or selection bias since both studies had only limited number of restenosis lesions. Histological examination of representative samples of the specimens set up in culture has shown that the characteristic feature of restenosis, intimal hyperplasia, may also be present in primary atherosclerotic lesions. Although the primary atherosclerotic specimens in general were less cellular with more dense collagenous stroma, overlap in the histology between the two types of lesions was not uncommon. Therefore it seems somewhat artificial to describe primary atherosclerotic lesions or restenosis lesions as distinct and separate histologic and pathologic entities.

Matrix Synthesis

Although vascular SMCs in culture are known to produce collagen and proteoglycans, there is no data available on human coronary SMCs from primary atherosclerotic and restenotic lesions. Previous studies have shown that modulation of the SMC phenotype to the "synthetic" state is associated with large increases in collagen (30 fold) and glycosaminoglycans (5 fold) (27,28). Our preliminary results show that the origin of the vascular SMC appears to affect the synthesis of collagen and glycosaminoglycan (summarized in Table 6). In identical culture conditions, coronary SMCs produced 50% more collagen and more than two times more glycosaminoglycans than SMCs from the media of the umbilical artery.

The enhanced matrix synthesis in the coronary cells may reflect a more specialized function of a SMC situated within an atherosclerotic or restenotic lesion. Previous studies have shown that the production of matrix components by vascular SMC is altered quantitatively and qualitatively in hypertension, atherosclerosis and angiogenesis (29). Based on our results, modulation of extracellular matrix components in vitro, may be a reflection of similar mechanisms operating in vivo.

The production of collagens by cultured SMC has been shown to be selectively and quantitatively increased (as a percentage of total protein synthesis) by cyclic stretching (30) and heparin treatment (31). Cells cultured from atherosclerotic aortas (32) or treated with growth factors (33) have also been shown to produce more collagen per cell, although collagen production relative to total protein synthesis was unchanged.

Previous studies have indicated that the synthesis of glycosaminoglycans and proteoglycans is complex and that alterations in cell density, serum and pH result in marked changes to both the amounts and proportions of GAG (34). The source of SMC also appears to affect the synthesis of glycosaminoglycans. In almost all studies, the SMC have been derived from thoracic aortas and muscular arteries (eg coronaries) have rarely been used. Two animal studies have shown a higher rate of GAG synthesis in cells cultured from experimentally induced atherosclerotic lesions or from atherosclerotic susceptible White Carneau pigeons (3-4x greater compared with the atherosclerotic resistant Show Racer breed) (36) and that most of the increase was due to an increase in sulfate GAG, especially dermatan sulfate. Although the amounts and proportions of the various types of GAG are changed at sites of human atherosclerotic lesion formation, there is limited data on human in-vitro synthesis, particularly in cells originating from atherosclerotic lesions. No studies have been reported

on SMCs originating from coronary lesions. Our results suggest that these cells behave differently in culture than cells from the media of umbilical arteries and therefore may be a more useful model for the study of atherosclerosis.

It is interesting to note the differences in proliferation rates and matrix synthesis between the coronary and umbilical SMCs in our study. Smooth muscle cell proliferation and synthesis of extracellular matrix are inversely related. Other examples are heparin and transforming growth factor-beta (TGF-B) which is the most potent of the growth factors in the control of extracellular matrix synthesis and increases the synthesis of chondroitin sulfate, the dominant extracellular matrix protein early in intimal hyperplasia, by 20-fold (37,38). However TGF-B may be a potent inhibitor of SMC growth in certain circumstances (39). Similarly, heparin has the dual action of inhibiting SMC proliferation and stimulating the synthesis of proteoglycans (14,40).

In summary, coronary SMCs from primary atherosclerotic and restenotic lesions can be successfully grown in culture. The specialized functions of proliferation and matrix production that are responsible for the development of these coronary artery lesions can be studied in this model. Preliminary data suggests that the cells originating from coronary plaques are particularly active in matrix synthesis. Future studies with this model will be aimed to determine the specific types of collagen and glycosaminoglycans produced by the umbilical and coronary SMCs and further evaluation of the differences in proliferation and synthetic functions between the two sources of SMCs.

Acknowledgement

We are grateful to Dr. Raoul P. Rooman, Janssen Research Foundation, Beerse, Belgium for helpful suggestions and critical review of the manuscript.

Table 1. Patient characteristics of serially passaged cell cultures.

Patient	Sex	Age	Procedure Type	Comments
1 (16)	M	48	Primary	
2 (19)	F	71	Restenosis	
3 (20)	M	69	Primary	growth limited to second passage
4 (45)	M	40	Restenosis	
5 (46)	M	66	Restenosis	
6 (52)	F	58	Primary	
7 (68)	F	49	Primary	
8 (72)	M	49	Primary	growth limited to third passage
9 (83)	M	50	Primary	
10 (84)	M	68	Primary	

Table 2. Population Doubling times (hrs)

Coronary			
	a) Primary	16P4	43
		52P4	65
		68P2	53
	b) Resten	19P4	43
		46P4	46
		Total	50±9
Control		7AP5	24
		7AP4	49
		7AP4	34
		7AP5	24
		Total	33±12

Table 3. Thymidine labelled cells (% of total cells)

Coronary			
	a) Primary	16P2	59.2%
		52P2	57.1%
	b) Restenosis	19P4	48.5%
		45P4	46.4%
		46P2	35.8%
		46P4	37.9%
Control (Umbilical)		8AP3	78.9%
		9AP2	63.4%

Table 4. H^3-Proline Incorporation

Type	Number	Protein	nm ^{35}H-Proline	nm Proline/ ug protein
Prim	16P2(3)	112	4.65	0.0416
	16P4(4)	31	0.56	0.0228
	52P2(3)	110	2.33	0.0410
	52P3(4)	44	0.50	0.0230
	68P2(4)	18	0.66	0.0380
Rest	19P4(4)	68	2.47	0.0363
	19P4(3)	13	0.54	0.0478
	45P4(3)	53	0.62	0.0117
	46P2(2)	53	1.94	0.0366
	46P4(3)	36	0.69	0.0196
	46P4(3)	10	0.47	0.0503
Cont	8AP2(3)	86	2.06	0.0239
	7AP4(3)	138	1.63	0.0118
	7AP4(4)	63	1.01	0.0205

Number() = cell line and the number of culture wells (replications) used in the experiment; nm = nanomoles; protein represents the total protein in the cell monolayer

Table 5. GAG Assay- Experiment #2 (September 1991)

Type	Number	Protein	moles S^{35} ($x10^{-7}$)	nmoles/ ug protein
Prim	52P3-1	71	9.03	12.70
	52P3-2	71	5.60	7.89
	84P4-1	68	14.1	20.74
	84P4-2	68	13.7	20.15
Rest	19P3-1	64	8.40	13.13
	19P3-2	64	9.27	14.48
	19P4-1	45	3.81	8.47
	19P4-2	45	3.84	8.53
	46P5-1	54	7.33	13.57
	46P5-2	54	5.75	10.65
Cont	86GSP5-1	85	3.45	4.06
	86GSP5-2	85	3.05	3.59
	86GSP5-1	75	5.95	7.93
	86GSP5-2	75	4.59	6.12

Table 6. Summary of Matrix Results

	Table 4	Table 6
Primary*	0.0333 ± 0.0096	15.37 ± 6.19
Restenosis*	0.0337 ± 0.0153	11.47 ± 2.62
Coronary[1]*	0.0335 ± 0.0124	13.03 ± 4.54
Control	0.0188 ± 0.0062	5.43 ± 2.00

[1] combined primary and restenosis results, * $p<0.05$ versus control

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Samenvatting

Met de introductie van percutane transluminale coronaire angioplastie (PTCA) leidde Andreas Gruentzig in 1977 het tijdperk van interventie cardiologie in (1). Het feit dat coronair angioplastie algemeen aanvaard is blijkt uit het grote aantal ingrepen (≥ 300.000 procedures in de Verenigde Staten in 1990), het toenemende aantal indicaties en het bestaan van een uitgebreide infrastructuur om de techniek te ondersteunen (inclusief de uitbreiding van catheterisatie laboratoria, opleidingsplaatsen en wezenlijke betrokkenheid van de industrie die zich toegelegd heeft op voordurende verbetering van geavanceerde röntgen afbeeldingstechnieken en angioplastie materialen). Naast de ballondilatatie werden in de afgelopen 5 jaar ook verschillende andere technieken geevalueerd als alternatieven of aanvullingen van PTCA zoals atherectomie, rotablator en verschillende vormen van laser therapie. Deze technieken werden ingevoerd vanwege de beperkingen van ballondilatatie, zoals vroegtijdige afsluiting en restenose binnen de eerste 6 maanden na de procedure. Deze beperkingen zijn blijven bestaan ondanks intensieve pogingen om farmacologische oplossingen hiervoor te vinden (2-5).

De eerste stent implantaties werden uitgevoerd in 1986 in Lausanne, Zwitserland, door Ulrich Sigwart en in Toulouse, Frankrijk, door Jacques Puel (4).

Stents zijn hulpmiddelen die binnen in het bloedvat worden aangebracht om mechanische ondersteuning van de vaatwand te verlenen. Een aantal types stents zijn ontwikkeld die verschillen in samenstelling, ontwerp en mechanisch gedrag. Volgens Dr. Philip Urban uit Geneve werden de eerste pogingen om buisjes in vaten te implanteren beschreven door Alexis Canel uit New York in September 1912 in het Amerikaanse maandblad "Surgery, Gynaecology and Obstetrics" (6). In deze vroege experimenten werden bij 7 honden korte glazen buisjes in the thoracale aorta geimplanteerd, 3 beesten ontvingen aluminium buisjes en bij 1 beest werd een verguld aluminium buisje ingebracht via een chirurgische incisie van de thoracale aorta. In 5 gevallen thromboseerde het buisje tussen 5 en 97 dagen na de implantatie en twee beesten verbloedden 8 en 11 dagen na de operatie. In 1969 paste Charles Dotter dit concept opnieuw toe toen hij ondoordringbare plastic buis prothesen implanteerde in normale arterieen (arteria femoralis en arteria poplitea) van honden. Zijn eerste pogingen faalden ten gevolge van thrombosering binnen 24 uur. Het succes verbeterde toen hij de plastic buisjes verving door roestvrij stalen spiralen en heparine infusies toediende gedurende 4 dagen na de implantatie (8). Verschillende endovasculaire stents worden thans klinisch en experimenteel geevalueerd. Veranderingen van ontwerp, het gebruik van legeringen en verkleining van de endoprothese heeft geresulteerd in een groot aantal verschillende types voor experimentele en klinische evaluatie.

De hoofdthema's van dit proefschrift zijn de coronaire stent en de reactie van de vaatwand na implantatie van de stent. In hoofstuk 2 wordt als inleiding tot de kwantitatieve angiografische analyses van de coronaire stent het idee om atheresclerotische stenoses te stenten als aanvulling op ballon dilatatie (angioplastie) alsmede het proces van reactieve intima hyperplasie ten gevolge van de combinatie ballon dilatatie met stent implantatie uitgewerkt. Dit alles gebaseerd op onze

dierexperimenten en humane veneuze vaatenten die 3 dagen to 10 maanden na het stenten chirurgisch werden verwijderd.

In hoofdstuk 3 en 4 worden enkele methodologische aspecten van kwantitatieve coronaire angiografische metingen in gestente bloedvaten belicht. De optimale methode om vroege en late angiografische resultaten na PTCA of stent implantatie te analyseren is nog niet vastgesteld. Hoewel contour detectie de belangrijkste analyse methode blijft, kan de toepassing hiervan worden belemmerd in geval van eccentrische laesies, vooral als de anatomie van de vaatwand verstoord is door een dissectie. Densitometrische analyse wordt dan aanbevolen als een alternatieve methode omdat deze methode theoretisch onafhankelijk is van de geometrische vorm van het bloedvat.

In hoofstuk 3 wordt een in vitro studie beschreven waarbij de invloed van 3 onlangs geintroduceerde coronaire stent types op de densitometrische meting van een bekende stenose in een plexiglas model word geanalyseerd. Het blijkt dat de roestvrij stalen stents (de Wallstent en de Palmaz-Schatz stent) slechts een geringe toename (≤8%) in het berekende oppervlak van de dwarsdoorsnede op de plaats van het minimale lumen veroorzaken. Echter, tantalum bevattende stents (Wiktor Stent) kunnen resulteren in een aanzienlijke overschatting van het dwarsdoorsnede oppervlak van de laesie bij densitometrische analyse, vooral als de contrast vloeistof verdund is of het vat niet goed is gevuld met contrastvloeistof.

In hoofdstuk 4 vergeleken wij de resultaten van de oppervlakte van de dwarsdoorsnede op de plaats van het minimale lumen verkregen door contourdetectie met densitometrie bij 19 patienten die coronair stenting ondergingen met de Wallstent. Hoewel een slechte correlaties tussen deze 2 methoden werd gevonden in eerdere studies na ballon dilatatie, werd door ons een veel duidelijkere correlatie waargenomen na stent implantatie, waarschijnlijk ten gevolge van vlakkere vaatwand contouren veroorzaakt door de stent en het zich vervormen van het gestente segment in een circulaire configuratie.

In de hoofdstukken 5-8 worden de vroege en late follow-up resultaten van de eerste klinische geimplanteerde coronair stent, de Wallstent, besproken. De 6 Europese centra die betrokken zijn bij het onderzoeken van deze techniek kwamen overeen om een angiografische "core laboratorium" voor kwantitatieve angiografische analyse op te richten in het Thoraxcentrum in Rotterdam om een objectieve beoordeling van de resultaten te waarborgen. Het Coronary Artery Analysis System (CAAS), dat zeer uitgebreid gevalideerd is, werd gebruikt voor alle kwantitatieve studies. Als aanvulling op de angiografische analyses waren klinische gegevens beschikbaar. Follow-up gegevens werden verkregen uit patienten statussen en gesprekken met deelnemende cardiologen.

In hoofdstuk 5 worden de resultaten van de eerste 117 "zelf expanderende" stents, geimplanteerd bij 105 patienten, beschreven. Deze studie vertegenwoordigt de eerste kwantitatieve follow-up studie van één van de nieuwe angioplastie technieken en is daarom van historisch belang. De belangrijkste bevinding van deze studie is het hoge occlusie percentage na stent implantatie (24%) hetgeen gedeeltelijk een gevolg bleek te zijn van patienten selectie, verschillende en veranderde antistollings protocollen in de verschillende centra en onervarenheid van de interventie cardiologen met de techniek.

Restenose werd vastgelegd in 14% van de doorgankelijke stents bij follow-up angiografie, hetgeen gunstig afsteekt bij vergelijkbare ballondilatatie studies.

Hoofdstuk 6 en 7 bevatten de gegevens van 256 patienten waarbij een stent geimplanteerd werd (308 laesies) tussen Maart 1986 en Maart 1990.

In hoofdstuk 6 hebben we angiografische voorspellers van restenose in de coronaire Wallstent geidentificeerd, gebruik makend van een relatief risico analyse; het gebruik van meerdere stents per laesie, te grote stents (geexpandeerde stent diameter meer dan 0,7 mm groter dan de referentie diameter), stent implantatie in veneuze bypass vaatenten en een hoge residuele diameter stenosis hadden een significante hoger risico op restenose. Aangezien enkele van deze factoren vermeden kunnen worden (implantatie van te grote stents, gebruik van meer stents, suboptimale dilatatie) konden aanbevelingen gedaan worden om het risico op restenose na stent implantatie te verminderen.

In hoofdstuk 7 wordt een uitgebreide analyse van klinisch resultaten, afsluitings en restenose percentages beschreven voor de gehele studie groep en ingedeeld naar het tijdstip van implantatie en het type bloedvat. Daar onze ervaring met stent implantatie de eerste in zijn soort was, evolueerden de indicatie voor implantatie en de behandeling van patienten na implantatie met toename van ervaring en kennis. Bij de eerste groep patienten (gedeeltelijk beschreven in hoofdstuk 5) werden voornamelijk stents geimplanteerd in kranslagvaten waarin eerder een restenose was opgetreden. De volgende groep bestond hoofdzakelijk uit patienten met primair vernauwde veneuze vaatenten en oorspronkelijke kranslagvaten die gestent werden als onderdeel van een "bail out" procedure na een gecompliceerde ballondilatatie. Alhoewel statistisch niet significant was er in deze groep een reductie van de thrombotische occlusies tijdens de opname in het ziekenhuis tot 12% gevolgd door een toename van het restenose percentage van 21% naar 32%. De palliatieve rol van stenting bleek ook duidelijk door de lage gebeurtenis vrije overleving voor zowel kransslagvaten (46% na 40mnd) als voor veneuze vaatenten (37% na 20mnd).

In hoofstuk 8 worden de afzonderlijke Thoraxcentrum ervaringen van stent implantatie in gestenoseerde veneuze vaatenten in detail beschreven. De toepassing van stent implantatie als behandelings mogelijkheid voor deze groeiende groep patienten die meestal niet gebaat zijn bij reoperatie of conventionele ballondilatatie, wordt in dit hoofstuk besproken. Alle in het Thoraxcentrum gestente patienten hadden ernstige angineuze klachten en de meerderheid werd beschouwd als hoog risico patient voor het ondergaan van een bypass operatie of ballon dilatatie. Afsluiting tijdens ziekenhuis opname kwam voor bij 10% van de patienten en trad gewoonlijk op bij acuut ischaemisch hartlijden of onderbreking van de antistollingstherapie. Hoewel het percentage restenose na stent implantatie hoog was (47%) en reinterventie frequent noodzakelijk was, kan coronaire stenting een effectieve behandeling zijn voor patienten die slechte kandidaten zijn voor alternatieve behandelings methoden.

In de hoofdstukken 9 t/m 11 worden de resultaten beschreven van onderzoek op coronair weefsel verkregen met behulp van de atherectomie catheter.

In hoofdstuk 9 worden de vroege en late resultaten onderzocht van directionele atherectomie ter

behandeling van restenose in een coronaire stent bij 9 patienten (10 procedures). Het verwijderde restenose materiaal werd bestudeerd wat betreft cel indentificaties, proliferatie activiteit en cellulariteit. Hiertoe werd gebruik gemaakt van een combinatie van lichtmicroscopie, electronenmicroscopie en immunohistochemische technieken. Deze resultaten werden vergeleken met een controle (niet gestente) groep patienten bij wie restenose weefsel was verwijderd 14-59 dagen na een eerdere ingreep (PTCA, atherectomie, laser). De angiografische resultaten toonden aan dat directionele atherectomie een effectieve behandelingsvorm is na restenose in de stent maar beperkt wordt door het optreden van re-restenosen. De weefsel studies toonden aan dat de gladde spiercel het belangrijkste celtype is dat aangetroffen wordt in restenose laesies onafhankelijk van het type van de eerdere ingreep, en dat proliferatie van gladde spiercellen een vroeg fenomeen is en 2 maanden na de procedure nog nauwelijk aantoonbaar is. Voorts blijkt dat de meerderheid van gladde spiercellen kort na de procedure het contractiele fenotype aannemen dat de cellulariteit afneemt in het verloop van de tijd. Er is echter een grote individuele variabiliteit.

In hoofdstuk 10 werd de afkomst van de gladde spiercellen in primaire coronaire laesies afkomstig uit een een kransslag vat na harttransplantatie aangetoond. Er werd gebruik gemaakt van een techniek (DNA fingerprinting) die gebaseerd is op verschillen in genetische samenstelling tussen donor en ontvanger van zogenaamde VNTR gen loci. Deze studies werden verricht op DNA dat geisoleerd werd uit een coronair atherectomie fragment en uit biopten van het donor myocard en bloed van de ontvanger na vermeerdering van het DNA door middel van een polymerase kettingreactie (PCR). De resultaten van de electrophorese bevestigden dat de intima laesies zoals gezien bij atherosclerose en restenose voornamelijk bestaan uit gladde spiercellen die hun oorsprong hebben in de vaatwand en niet afkomstig zijn uit de circulatie.

In hoofdstuk 11 worden de voorlopige kweek resultaten van gladde spiercellen uit coronaire atherectomie fragmenten (inclusief restenose laesies in gestente arterieën) beschreven en vergeleken met gladde spiercellen die geisoleerd werden uit de media van arterien uit de menselijke navelstreng. De belangrijkste bevindingen van deze studie waren dat uit een minderheid van de lesies seriële passage mogelijk was en dat deze cellen een lager proliferatie percentage (50%) vertonen maar een hogere graad van extracellulaire matrix componenten, collageen (50%) en gesulfateerde glycosaminoglycanen (≥2x) dan de navelstreng gladde spiercellen. Dit celkweek systeem blijkt een nuttig model te zijn bij de bestudering van processen zoals atherosclerose en restenose.

Het doel van dit proefschrift is tweeledig. De interventie cardiologie is nu het post-ballon dilatatie tijdperk binnengetreden en we worden geconfronteerd met uitdagingen die 15 jaar geleden niet konden worden voorzien. We zijn getuige geweest van een explosie van alternatieve technieken voor ballonangioplastie zoals diverse types stents, tenminste 3 verschillende atherectomie catheters en een overvloed van verschillende laser catheters. Klinische acceptatie van één van deze nieuwe technieken wacht op uitgebreide wetenschappelijke evaluatie. Op dit moment kan dit alleen bewerkstelligd worden door het gebruik van kwantitatieve coronaire arteriografie.

Ondanks het feit dat andere evaluatie methoden in de toekomst toegepast zullen worden (bijv. intravasculaire echografie) moeten toekomstig onderzoek zich niet beperken tot de technische aspecten van deze meer eisende en meer gecompliceerde technieken, maar ook gericht zijn op het verwerven van nieuwe inzichten in de processen die het lange termijns resultaat van coronaire interventies kunnen beinvloeden.

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