Mechanisms, Pathology and Therapeutic Interventions of Restenosis

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Restenosis is a particular problem in small vessels following a successful percutaneous transluminal coronary angioplasty remains the main obstacle to this technique for myocardial revascularisation. The occurrence of restenosis, which is now known to be caused by both vessel remodelling and neo-intimal hyperplasia, might be reduced in the future by a combined mechanical and pharmacological approach. Despite intensive investigation in this area, no pharmacological therapy has yet been found to be useful in preventing restenosis after conventional balloon angioplasty. Though dramatic improvements in catheter and stent technology, in-stent restenosis continue to hamper initial procedural success in patients undergoing coronary intervention. With the advent of drug eluting stents, opportunities for even greater long term success appears to be a reality.

The primary cause of cardiac morbidity and mortality in developed countries is ischemic heart disease (IHD). The incidence of this disease is virtually all due to atherosclerosis and IHD is also the most prevalent disease in the industrialized world and is the leading cause of death in many parts of the world. The main aim of the therapy is to provide revascularization to the ischemic myocardium. The publication of the Bypass Angioplasty Revascularisation Investigation (BARI) data confirms and consolidates pervious findings that balloon angioplasty and bypass surgery are equivalent in terms of mortality and major complications over a mid-term follow-up period. In about 20 years, percutaneous transluminal coronary angioplasty (PTCA) has become the most commonly used method world wide for revascularisation. Restenosis in the months following a successful PTCA remains the main limitation to this technique for myocardial revascularisation. Restenosis after PTCA is a clinical problem associated with major ischemic events or repeat interventions in 20-50% of the treated patients. Restenosis occurs in 25-45% of all patients within 6 months and attempts to pharmacologically prevent or re-

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Stent implantation represents a major step forward since the introduction of coronary angioplasty. Coronary stenting has made a significant difference in percutaneous coronary revascularisation, techniques, since it provides an effective treatment for procedural complications and prevents to some degree the incidence of restenosis. Presently, most interventional cardiologist needs are well covered due to the availability of many different stents in the market. Numerous stents are now in use: self expanding and balloon expandable stents, stainless steel stents, tan-
talum stents, flexible and articulated stents, antithrombotic coating stents in particular hirudin/liprost combination\textsuperscript{17}, antiproliferative coating stents in particular sirolimus and paclitaxel have shown the greatest promise in early clinical trails\textsuperscript{14}, intracoronary radiation therapy and glycoprotein Iib/IIla inhibitors\textsuperscript{18}. Implantation of coronary stents is not free of complications. In addition to wall injury at the site of stent deployment, which provides a powerful stimulus to platelet activation and thrombus formation, the surface of the stent itself constitutes a thrombogenic foreign body. Thus, without treatment a high rate of early stent thrombosis may be expected. Further more, together with the impact of the arterial wall injury, a multifactorial process is initiated, leading to neointimal hyperplasia and restenosis\textsuperscript{17}. Although systemic administration of antirestenosis drugs has not yet been tested to prevent restenosis after coronary stenting it is very likely that pharmacological inhibition of neointimal hyperplasia within coronary stents will take advantage of local delivery techniques. In addition to local drug delivery catheters that are available, the stent itself may be coated with polymers and serve as a platform for drug delivery. Future trends in stent design will involve multicellular designs and increased flexibility with the aim of reducing stent-vessel wall interactions and restenosis\textsuperscript{18}. The continued attractiveness of PTCA, as an alternative to medical treatment or bypass surgery for patients with coronary artery disease will depend upon our ability to control the restenotic process.

**MECHANISMS OF RESTENOSIS**

**Neointimal hyperplasia:**

In response to experimental arterial injury, medial smooth muscle cells (SMCs) shift from a contractile to a synthetic phenotype, proliferate, migrate and produce large amounts of extracellular matrix\textsuperscript{19} Multiple factors lead to the activation of SMCs immediately after arterial injury. Expression of nuclear oncogenes, which are early markers of SMC activation are detectable as soon as 30 minutes after injury\textsuperscript{20}. Induction of c-fos, c-jun and c-myc protooncogenes is one of the earliest transcriptional events associated with growth factor stimulation and the increased expression of these genes is a transient response to mitogenic stimulation persisting at most, for a few hours after exposure to growth factors\textsuperscript{21}. It has been recently demonstrated that the distribution of c-fos and c-jun products after arterial injury is concentrated in SMC nuclei\textsuperscript{20}. The corresponding oncoproteins bind to a specific DNA sequence to target genes to stimulate their transcription and are involved in the G\textsubscript{1} phase of the cell cycle\textsuperscript{22}. At least 20 to 40% of medial SMCs are activated and enter the cell cycle between 24 hours and 3 days after balloon denudation\textsuperscript{23}. These cells then migrate to the intima through breaks in the internal elastic membrane. Many of these neointimal cells continue to proliferate for several cycles but nearly half of the migrating cells do not synthesise DNA\textsuperscript{23}. This growth response leads to development of a neointimal thickening also known as neointimal hyperplasia\textsuperscript{24}.

**Arterial remodelling:**

There is increasing experimental evidence that neointimal hyperplasia is not the sole mechanism leading to lumen renarrowing after angioplasty, and that arterial remodelling also plays a major role in this process\textsuperscript{25}. In the hypercholesterolemic rabbit model, Kakuta et al.\textsuperscript{26} showed that compensatory enlargement of the vessel (increase in internal elastic lamina area) occurs in the weeks following experimental angioplasty. Surprisingly, restenosis was not related to neointimal formation but to a lack of compensatory enlargement or even to some degree, vessel constriction. Vascular remodelling is thus able to limit the effect of neointimal formation on the chronic lumen diameter, and difference in vascular remodelling not differences in intimal formation, account for restenosis in this model.

Neointimal hyperplasia is not the sole mechanism of restenosis in humans. There is intracoronary ultrasound evidence that vascular remodelling also occurs after angioplasty in humans\textsuperscript{26,27}. Studies by Mintz and colleagues\textsuperscript{26,27} suggested that most of the late lumen loss after conventional balloon angioplasty was caused by arterial remodelling and not neointimal formation. The mechanism of restenosis within coronary stents is not completely understood but preliminary intravascular ultrasound studies\textsuperscript{27} suggest that coronary stenting effectively prevents vessel constriction and that most of the late lumen loss occurring after stent implantation is due to plaque growth related to neointimal hyperplasia. The mechanism of restenosis within coronary stents is different from that of restenosis after conventional balloon angioplasty. This implies that a treatment shown to be ineffective in preventing restenosis after PTCA will not necessarily be ineffective in preventing restenosis after coronary stenting.

**PATHOLOGY OF IN-STENT RESTENOSIS**

Stent implantation lowers restenosis rates. However, stent deployment results in early thrombus deposition and acute inflammation, granulation tissue development and ultimately SMC proliferation and extracellular matrix syn-
thesis. The reaction of the vessel provoked by stenting is subdivided in three phases, which are described below.

**Thrombotic phase:**

Vessel injury and mechanical irritation provoked by stent implantation initially lead to a bursting activation and adhesion of thrombocytes, generating a thrombus on the side of vessel denudation. Platelets bind to the subendothelial area and form pseudopodies reaching the connective matrix. Additionally, the steel surface of the stent enhances thrombus formation by activating the blood coagulation cascade, the complement system and the adhesion of fibrinogen to the stent surface. Twenty-four hours after stent implantation, a fibrin containing layer accumulates around the adhesive thrombocytes. The vessel injury also causes an enhanced expression of intra cellular adhesion molecule 1 (ICAM-1) on SMCs. ICAM-1 is an adhesion protein for the recruitment of cells of the immune system.

**Recruitment phase:**

In the recruitment phase, an intense infiltration of monocytes and cells of the immune system in the thrombocyte-fibrin layer occurs. Adhesion of leucocytes is thought to be the second inducer of neointima proliferation after platelet aggregation at the injury site. The leucocyte and platelet-containing layer produces large quantities of proliferation stimulating substances with main platelet origin, such as Platelet Derived Growth Factor β (PDGF-β), β-fibroblast growth factor (β-FGF) and thrombin. In the thrombus of the injured vessel, activated macrophages release progression factors (i.e. βFGF, IL-1) that enhance the platelet-induced SMC migration and proliferation. Additionally leucocyte and platelet activation synergistically enhance fibrin production. The release of neutrophil granule contents of thrombus attached neutrophils promotes more platelet aggregation and degranulation, as well as fibrinogen receptor expression. Thrombus attached cells of the immune system also express receptors for coagulant proteins, suggesting intimate involvement in the clotting cascade.

**Healing phase:**

During this stage, actin positive cells colonize the thrombus, progressively spreading and reabsorbing the remaining thrombus until it is gone and replaced by neointimal cells. Proliferating SMCs then utilize the thrombus as a biodegradable proliferation matrix. Migration and proliferation of SMCs cause additional degradation of collagen in the extracellular matrix, resulting in a reduced matrix content in restenotic areas than in non-restenotic vessels.

The resulting neointimal thickness induced by the neointimal cell layer is much larger than the original thrombus and occasionally causes a restenosis phenomenon.

**PHARMACOLOGICAL PREVENTION OF RESTENOSIS**

A large number of clinical trials have examined whether systematically administered pharmacological agents reduce the risk of an angiographic restenosis. The overwhelming majority of these clinical pharmacological studies have failed to show a significant reduction in the incidence of restenosis in humans (Table 1).

**Antiplatelet agents:**

Platelet adhesion and activation is an important step in vascular healing during the first days after angioplasty. A number of studies showed a reduction in the incidence and severity of recurrent stenosis in the atherosclerotic rabbit model, in animals treated with aspirin and dipyridamole. In humans, the use of aspirin, ticlopidine, thienopyridine, antagonists and prostacyclin analogues have been studied. More recently, the glycoprotein receptor GP IIb/IIIa antibody has been shown to reduce clinical events 6 months after PTCA in the EPIC trial. Angiographic studies are currently being performed to analyze the impact of these very potent antiplatelet agents on restenosis.

**Anticoagulants:**

Both nonfractionated heparin and newer low molecular weight derivatives have demonstrated antiproliferative activity in animal models such as rat and the rabbit. However, in humans, short term intravenous heparin and 1 to 3 month treatment with enoxaparin has been shown to be ineffective in preventing restenosis. Recent clinical trials have evaluated the more powerful antithrombin hirudin which inhibits experimental restenosis but does not appear to be effective in humans.

**Growth factor inhibitors:**

Growth factor inhibitors such as trapidil and angiopeptin were shown to be effective in experimental models of restenosis. Angiopeptin, a synthetic cyclic octapeptide analogue of somatostatin, has been shown to reduce neointimal hyperplasia in several different animal models of angioplasty. The mechanism of this effect is unknown but is thought to be related to a local inhibition of growth factors responsible for SMC activation. Two large clinical trials one in Europe and one in US have recently reported that angiopeptin had no effect in preventing angiographic restenosis.
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Lipid-lowering agents:

Statins, which block the synthesis of cholesterol by inhibiting the enzyme HMG-CoA reductase, have been shown to reduce intimal hyperplasia after balloon angioplasty in rabbits but it has been recently published that there is no benefit of treatment in the prevention of restenosis in humans. Probucol, previously used as a lipid-lowering agent also has powerful antioxidant properties, has been shown to be effective in reducing the rate of restenosis when administered 4 weeks before PTCA. Clearly probucol is the first therapy to be effective in preventing restenosis after conventional balloon PTCA.

Angiotensin converting enzyme (ACE) inhibitors:

Based on the demonstration that angiotensin II plays an important role in the control of SMC growth, a potential role for ACE inhibitors in the limitation of neointimal proliferation has been suggested. After initially positive results in experimental models, three large clinical studies examining cilazapril and fosinopril failed to show any significant impact on restenosis rates. A strong influence of the ACE insertion/deletion (I/D) gene polymorphism was identified after coronary stenting, while no effect was found after conventional balloon angioplasty.

Other agents:

Many other agents have been evaluated as potential inhibitors of restenosis. Recently the results of the ACCORD study suggested by molsidomine, a direct nitric oxide donor, may significantly reduce the risk of angiographic restenosis. These results must be regarded as preliminary and need to be confirmed as the trial was not blinded. Preliminary clinical studies suggest that there is a potential benefit in using the c-myc antisense strategy to prevent SMC proliferation and collagen expression which is closely linked to remodelling. However, these therapeutic strategies need to be validated in large trials.

LOCAL DRUG DELIVERY TECHNOLOGY FOR PREVENTING RESTENOSIS

Local administration of pharmacological agents directly to the site of coronary intervention has been advocated as a means of concentrating drug in the injured arterial tissue to inhibit restenosis. Several catheters have been designed for specific delivery of drugs or gene products. Unfortunately drug delivery efficiency and long-term retention remain problematic. In an effort to overcome the limitations of local drug delivery associated with the use of catheters, drug-loaded stents have been developed. Loading of such stents is achieved through either drug absorption (incorporation into matrix) or drug adsorption (surface layering). Stent implantation represents a major step forward since the introduction of coronary angioplasty. Metallic stents are useful for suboptimal PTCA results or threatened closure and can reduce restenosis in de novo lesions. However, they are permanent devices that are used to treat a short-term problem and have only limited potential for local drug delivery. The intrinsic thrombogenicity and permanent stimulation to injured vessel wall tissue of all the current available metallic stents may result in hospital events such as thrombosis, (subacute coronary closure, emergency bypass surgery, hemorrhagic complications, pseudoaneurysm or even vessel perforation) and restenosis. To overcome these limitations and provide a scaffold for the remodelling vessel as well as a vehicle for sustained local drug delivery, bioabsorbed stents have been proposed as an alternative.

Coated stents:

Systemic application of anticoagulative drugs have failed to suppress restenosis sufficiently. One reason is that the drug concentrations required locally to achieve SMC growth inhibition or thrombus reduction are considerably higher than those achieved without a significant bleeding risk under clinical conditions. Coated stents serve as a vehicle for local high dose and site specific drug delivery. Local drug delivery via stents coated with immobilized drug or coated with a drug releasing polymer matrix offers the possibility of focal therapeutic drug effect within target tissues without serious side effects arising from systemic drug administration. An intact vessel prevents adhesion and aggregation of thrombocytes by the endothelial release of prostacyclin (PGI₂), nitric oxide and glycosaminoglycan proteins (endogene heparin analog). Therefore, a stent coating has to mimic endothelium in the injured vessel after stent implantation to prevent adhesion of proteins, local thrombin formation, aggregation of thrombocytes and to suppress excessive neointima formation. Coating serves as a carrier for local drug delivery, acting as an endothelial like barrier between blood and the metallic surface of the stent.

Selection of the coating material:

The coating substance used as a drug vehicle must be biodegradable, biocompatible and provoke no or only minimal inflammatory reaction. Initial trials conducted with polyurethane as coating polymer found that the non-biodegradable polymer induces severe inflammatory response in the
stented area. Polycondensed derivatives of lactic acid and glycolic acid as co-(poly-glycolic-lactic acid) or monomer [poly-LD-lactic acid (PLA)] are available as polymers of different molecular mass. The severity of the foreign body reaction depends on the degradation time of the polymer: with increasing degradation times, the immune response reduces. PLA degrades by 10% in 30 days. It is non-toxic, since it degrades to lactic acid and is metabolised in the citric acid cycle to CO₂. The continuous degradation of the PLA surface releases concomitant drugs embedded in the coating and prevents permanent adhesion of blood plasma proteins.

Great attention has to be given to the fact that the stent surface does not crack or partially flake off. The resulting cracks and exposed layers represent a stimulus for enhanced platelet and fibrinogen deposition. Based on a specific coating technique, the polymer is well fixed on the stent surface without rupturing or flaking when the stents are crimped and expanded.

FUTURE PERSPECTIVES

Although advances in catheter and stent design have dramatically improved procedural success and thrombosis rates following coronary intervention, the development of in-stent restenosis provokes recurrence of symptoms in 10 to 50% of patients treated with conventional stainless steel stents. After more than a decade of development, recent breakthroughs in polymer science and local drug delivery system have generated tremendous excitement about the potential application of these stents in the prevention of restenosis.

The role of drug eluting stents in many clinical scenarios has not yet been examined. Several trails are carefully enrolling patients to determine the potential impact of drug eluting stents in the treatment of in-stent restenosis. Despite the many unanswered questions surrounding the use of drug-eluting stents, there is a little doubt that the findings to date mark an unprecedented advance in the percutaneous management of patients with symptomatic coronary disease.

As we gain more experience with the technology, incorporating the use of drug eluting stents into our daily practice, we may continue to gain insights into the underpinnings of restenosis and into potential future strategies with which we may combat them. Due to these ongoing studies, recent breakthrough in technology, it is imperative for cardiologist to stay current with the latest information for the benefit of their patients.

REFERENCES