Platelets and Restenosis
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Restenosis is currently the major limitation of percutaneous transluminal coronary angioplasty (PTCA). Factors such as elastic recoil, migration of vascular smooth muscle cells from media to intima, neointimal proliferation and vascular remodeling underlying the restenotic process. Presently there is no effective therapy available for restenosis. The role of platelets in the development of thrombosis and abrupt closure after PTCA is well recognized. However, the effects of platelets in PTCA extend well beyond the early phase. Although antiplatelet agents such as glycoprotein IIb/IIIa antagonists have been reported to reduce target vessel revascularization, major unresolved controversies still exist. This report reviews the potential role of platelets in restenosis. Various drugs, successfully tested in experimental studies and in a small number of human studies, that inhibit the effect of platelets on the restenotic process are also reviewed. (J Am Coll Cardiol 2000;35:555–62) © 2000 by the American College of Cardiology

The utility of percutaneous transluminal coronary angioplasty (PTCA) has been established since the technique was reported in 1978 (1). Presently the primary limitation of PTCA appears to be restenosis (defined as >50% diameter stenosis on follow-up angiography), seen in up to 30% to 40% of patients (2). Restenotic lesions are histopathologically different from primary atherosclerotic lesions: comparatively, significantly greater smooth muscle cell (SMC) hyperplasia and less densely layered collagen and degenerating tissue have been observed in restenotic lesions (3). Restenosis is considered to occur over three phases: phase I = elastic recoil within 24 h; phase II = mural thrombus formation and organization within two weeks; and phase III = neointimal proliferation and extracellular matrix synthesis within three months (2). Inadequate compensatory enlargement of a vessel after angioplasty (inadequate vascular remodeling) is also considered important in restenosis (4). Inadequate arterial remodeling may be reduced to an extent by coronary stenting (5). However, a permanent metallic prosthesis does not reduce neointimal proliferation, which is considered to be the primary mechanism of restenosis after stenting (6).

PLATELETS AND PATHOPHYSIOLOGY OF RESTENOSIS

Platelet activation. In an animal model of angioplasty, platelet deposition was noted to occur immediately after injury and was undetectable by seven days; SMC migration and proliferation in media have been noted as early as 36 h after arterial injury (7) and occur within seven days (8). Platelets were found to relate directly to intimal proliferation after arterial injury, and severe thrombocytopenia inhibited intimal thickening, an effect that correlated with the degree of thrombocytopenia (9). After arterial injury, platelets rapidly adhere to the site of injury by several adhesion receptors: thromboxane A₂ is generated, changes in the glycoprotein (GP) IIb/IIIa complex occur, which then bind to fibrinogen, subsequently leading to platelet aggregation and activation (10). Activated platelets release, among other factors, platelet-derived growth factor (PDGF), a potential SMC mitogen (11). Figure 1 summarizes the potential platelet-derived factors that are known to play a role in neointimal proliferation after balloon injury.

Role of PDGF. Platelet-derived growth factor is the most important growth factor released by activated platelets. The association between PDGF and vascular SMC proliferation has been demonstrated in animal experiments (12) in which the rise and augmented levels of PDGF-B after arterial injury correlated with neointimal cellular proliferation. Besides inducing proliferation, the primary effect of PDGF on vascular SMCs could be the induction of migration, as PDGF is the strongest reported chemoattractant for vascular SMCs (13). Platelet-derived growth factor is postulated to act through autophosphorylation of PDGF receptor, leading to activation of tyrosine kinase and phosphorylation of exogenous proteins (12,14), culminating in new deoxyribonucleic acid (DNA) synthesis. Mitogen-activated protein kinase pathways may also be involved (15).

Effect of other platelet-derived factors. The expression of transforming growth factor-beta (TGF-beta) is reported to be increased in atherosclerotic vessels of rabbits after balloon injury (16). Also, direct transfer of TGF-beta₁ gene into porcine arteries resulted in intimal hyperplasia and increased extracellular matrix production (17). Serotonin and thromboxane A₂ are released by activated platelets and promote
SMC proliferation, both alone and synergistically when combined together (18). However, in human trials, thromboxane A2 receptor antagonists did not show a beneficial effect on restenosis (19). P-selectin is a glycoprotein stored in alpha-granules of platelets, expressed on activation of platelets. It is implicated in platelet-leukocyte interactions, and decreased neointimal formation has been demonstrated in P-selectin-deficient mice (20). Histamine released from activated platelets at the site of vascular injury has been postulated to induce intimal hyperplasia through H1 receptors (21). Interleukin-1 derived from platelets increases production of interleukin-6 and interleukin-8, which are important mediators of inflammation at the site of vascular injury (22). Although platelet-activating factor induces proliferation in SMC cultures (23), it has been demonstrated to have no influence on restenosis (24).

Platelet function and size on restenosis. Platelet reactivity and size also appear to influence restenosis after arterial injury. By stepwise regression analysis, abnormal platelet reactivity (defined as platelet reactivity index $>1.07$) before PTCA was reported as an important factor in the development of restenosis (30); restenosis at six months was seen in 25% of patients with normal platelet reactivity, 50% of patients with mildly abnormal platelet reactivity and 60% of patients with frankly abnormal platelet reactivity (30%) for the entire group. A significant positive correlation between mean platelet volume and restenosis has also been reported, with a relative odds ratio of 10.2 for the development of restenosis if the mean platelet volume was in the upper rather than the lower quartile (31).

Effect of exposure to contrast agent and artificial surface. Platelet function can be influenced by the type of contrast agent used. In an in vitro study, nonionic contrast medium showed an increased incidence of platelet degranulation; this event could contribute to restenosis by promoting increased local release of procoagulant molecules and PDGF (32). Increased platelet activation, demonstrated in in vitro experiments using stents, is postulated to occur because of exposure to artificial surfaces and shear forces of stent meshes (33). In human studies after intracoronary stenting, increased platelet activation has been found to correlate significantly with restenosis (6). In this study, a significant increase in GPIIb/IIIa ligand binding was seen in patients with restenosis as compared with patients without restenosis. Patients with restenosis also showed higher P-selectin expression and GPIIb/IIIa activation.

Platelets and high risk patients. Certain patient groups, such as those with diabetes mellitus, chronic renal failure or hyperlipidemia, are at a higher risk for restenosis. Interestingly, abnormal platelet function has been noted in all of these disorders. Elevated platelet counts and decreased nitric oxide synthase activity in platelets have been demonstrated in patients with diabetes (34,35). Increased platelet turnover and activation occur in patients with renal failure who have had long-term hemodialysis (36). Hyperlipidemia is associated with platelet hyperactivity (37), and antilipemic therapy has been shown to improve platelet function (38). The role of platelets in increasing restenosis in these high risk patient groups merits investigation.

Platelets and remodeling. Arterial remodeling is an important mechanism of restenosis (4), particularly in nonstent interventions. Platelets can adversely affect the process of remodeling after PTCA. Long-term treatment with PDGF

**Figure 1.** Schematic representation of platelet-derived growth factors involved in neointimal proliferation after vascular injury. $TxA_2 = \text{thromboxane A}_2$; $vWF = \text{von Willebrand factor}$. 

**Abbreviations and Acronyms**

- AS-OLN = antisense oligonucleotides
- DNA = deoxyribonucleic acid
- GP = glycoprotein
- mRNA = messenger ribonucleic acid
- PDGF = platelet-derived growth factor
- PTCA = percutaneous transluminal coronary angioplasty
- SMC = smooth muscle cell
- TGF = transforming growth factor

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has been shown to induce negative remodeling in porcine coronary arteries (39), and increased PDGF activity has been implicated in impaired vascular remodeling contributing to restenosis after coronary atherectomy in humans (40). Activation of tyrosine kinase of the PDGF receptor (39) and PDGF-induced increased cytosolic calcium concentration (41) are some of the mechanisms considered to be responsible.

**ANTIPLATELET THERAPY FOR DECREASING RESTENOSIS**

Various modalities of treatment, such as brachytherapy (42,43), inhibition of proto-oncogenes (44) and viral vectors for arterial gene transfer (45), have been reported to decrease SMC proliferation and reduce restenosis. Many antiplatelet agents have also been successful in reducing SMC proliferation after arterial injury.

**ANTIPLATELET COMPOUNDS**

Several antiplatelet agents have been investigated for the prevention of restenosis. In a randomized trial, the combination of aspirin and dipyridamole was found to have no significant effect on restenosis (46). Likewise, ticlopidine did not show a beneficial effect in reducing target vessel revascularization or restenosis in the Intracoronary Stenting and Antithrombotic Regimen (ISAR) trial (47). Recently, a new antiplatelet agent, clopidogrel, has been reported to be a safe alternative to ticlopidine (48); its influence on restenosis is not yet known. The utility of a monoclonal antibody to prevent platelet aggregation was first reported by Coller (49). The murine/human chimeric monoclonal antibody fragment, c7E3 Fab, has been shown to inhibit thrombin formation, reduce the need for target vessel revascularization by inhibiting activated platelets after PTCA (50,51) and decrease the frequency of ischemic events after coronary stenting (52). The favorable effect of c7E3 Fab in reducing the need for target vessel revascularization after PTCA appears to be sustained at three years (53). Decreased thrombin generation by c7E3 Fab and its cross reaction with the vitronectin receptor, αβ3, could contribute to a reduction in the need for target vessel revascularization (54). A similar finding has been reported in in vitro experiments (55); c7E3 Fab inhibited thrombin generation by 45% to 50%, which was similar to the magnitude of inhibition obtained by combining two different antibodies with properties of GPIIb/IIIa inhibition and αβ3 inhibition, respectively. The authors (55) conclude that c7E3 Fab significantly inhibited thrombin generation through both GPIIb/IIIa and αβ3 blockade. The αβ3 integrin also mediates SMC migration (56) and proliferation (57) in response to vascular injury. Inhibition of αβ3 by nonselective antagonists was reported to inhibit neointimal proliferation in animal experiments (58).

Besides GPIIb/IIIa and αβ3 blockade, c7E3 Fab decreases expression of the leukocyte integrin, Mac-1 (CD11b/CD18), after PTCA (59). After PTCA, expression of Mac-1 by circulating leukocytes was more marked in patients who subsequently developed restenosis (60). Administration of an antibody against Mac-1 to decrease adhesion and migration of leukocytes resulted in decreased intimal hyperplasia after balloon injury in rabbits (61). Further, blocking adhesion of Mac-1–bearing cells to fibrinogen and intercellular adhesion molecule–1 by c7E3 Fab at the site of vascular injury may lead to a lower injury response (62). However, despite a multitude of experimental evidence, the role of c7E3 Fab in reducing restenosis still remains controversial. The beneficial effects of c7E3 Fab in reducing the need for target vessel revascularization, as reported in the Evaluation of IIb/IIIa platelet receptor antagonist 7E3 in Preventing Ischemic Complications (EPIC) trial (with angiographic data lacking), were not reproduced in the subsequent Evaluation of PTCA to Improve Long-Term Outcome by c7E3 GPIIb/IIIa receptor blockade (EPILOG) (63) and Chimeric 7E3 AntiPlatelet in Unstable angina REfractory to standard treatment (CAPTURE) (64) trials, although the recent Evaluation of Platelet IIb/IIIa Inhibition in STENTing (EPISTENT) analysis has reported a lower target vessel revascularization rate in the subgroup of patients with diabetes treated with stents plus abciximab as compared with the patients with diabetes treated with stents alone (65). In the Evaluation of Reopro And Stenting to Eliminate Restenoses (ERASER) trial (66), which is the only study of c7E3 Fab using intravascular ultrasound, no benefit in restenosis was noted. In a recent study in nonhuman primates, despite an adequate antiplatelet effect (documented by markedly prolonged bleeding time), no beneficial effect of c7E3 Fab on neointimal proliferation, as compared with placebo, could be demonstrated in both angioplasty- and stent-treated segments (67).

Another antiplatelet agent, cilostazol, has demonstrated decreased restenosis in humans. This orally administered phosphodiesterase type III inhibitor decreases platelet activation by increasing cyclic adenosine monophosphate concentration within platelets; cilostazol also directly inhibits SMC proliferation (68,69). Other antiplatelet agents that inhibit platelet adhesion have also been reported to decrease neointimal proliferation. Nitric oxide donors, which have an inhibitory effect on platelet aggregation (70), and aurintricarboxylic acid, which interferes with the von Willebrand factor–platelet GPIIb/IX interaction, have shown decreased neointimal proliferation after balloon injury (71).

**Platelets as vehicles for local drug delivery.** Platelets are biodegradable and exhibit target localization at the site of arterial injury. Attempts are being made to use these properties to deliver drugs locally to reduce restenosis. The platelet membrane can be reversibly permeabilized by electroporation. By using platelets loaded with iloprost (a prostacyclin analogue), significant reductions in intimal area and intima/media ratios after angioplasty have been dem-
This effect was greater than that with local delivery of free iloprost. Similar in vitro results have been reported using iloprost-encapsulated platelets (73). Inhibition of thromboxane A2 produced by activated platelets could also contribute to the effect of iloprost.

**ANTI-PDGF AGENTS**

Many strategies directed at inhibition of PDGF-induced SMC proliferation have been successfully tested in vitro and in vivo (Table 1). These drugs are postulated to act at different stages of PDGF-induced SMC proliferation (Fig. 2).

**Drugs inhibiting PDGF release from platelets.** Naftidrofuryl, used in the treatment of peripheral vascular disease, and indomethacin have been reported to independently inhibit platelet aggregation and PDGF release and show an enhanced effect when combined in vitro (74). In a small study designed to assess the efficacy of acetylsalicylic acid as an anti-PDGF agent, platelet-rich plasma obtained from healthy volunteers 12 h after oral acetylsalicylic acid administration showed inhibition of collagen-induced release of PDGF; however, no effect on the release from alpha-granules in serum was demonstrated (75). The significance of this observation is not reported. In clinical studies, acetylsalicylic acid did not reduce the restenosis rate after angioplasty (46).

**Drugs acting on the PDGF receptor.** The structure of the PDGF receptor includes a ligand-binding domain in the extracellular portion and a tyrosine kinase region in the intracellular portion. Drugs acting on the PDGF receptor may interfere with binding of PDGF with its receptor or act through inhibition of tyrosine kinase. Trapidil (triazolopyrimidine), a PDGF antagonist, exerts its effect by competitive PDGF receptor blockade (76). Trapidil is also reported to interfere with the regulation of PDGF-beta receptor transcript levels (77); in addition, trapidil is a thromboxane A2 antagonist (78).

A meta-analysis of trapidil has shown that trapidil could reduce restenosis by 40% to 53% (79). A large study of trapidil using intravascular ultrasound (Trapidil vs. Placebo to Prevent In-Stent intimal hyperplasia [TRAPIST] study) failed to demonstrate any beneficial effect on restenosis (80). Another approach has been the application of antisense oligonucleotides (AS-OLN) in the prevention of restenosis. These oligonucleotides prevent messenger ribonucleic acid (mRNA) translation into protein by hybridizing with the targeted mRNA sequence (81). Use of AS-OLN may be associated with toxic effects such as bone marrow suppression and inhibition of synthesis of intracellular proteins (82). Use of AS-OLN against the PDGF receptor was first reported by Sirois et al. (83); local perivascular administration in rat carotid arteries of AS-OLN to the PDGF-beta receptor resulted in nearly complete inhibition of expression of PDGF-beta receptor. Intimal thickening was reduced by almost 80%, and precise correlation was found between neointimal proliferation and PDGF-beta receptor expression.

![Figure 2. Brief representation of possible mode of action of PDGF on SMC leading to restenosis. Numbers in parentheses indicate sites of action of various anti-PDGF drugs, as listed in Table 1.](image-url)
Angiopeptin, a long-acting somatostatin analogue, is reported to inhibit PDGF-induced vascular SMC proliferation (14). This compound activates specific protein tyrosine phosphatases that deactivate the phosphorylated PDGF receptor proteins (84). Tyrosine kinase inhibitors have also been investigated in the therapy of restenosis. PD 089828, which belongs to the 6-aryl-pyrido-(2,3-\(\beta\))-pyrimidines, has demonstrated long-lasting and reversible inhibition of PDGF-stimulated DNA synthesis and PDGF-induced migration and proliferation of vascular SMCs (85). Other tyrosine kinase blockers such as tyrophostins AG 1295 and AG 1296 also show selective inhibition of PDGF (86). Troglitazone, an insulin-sensitizing agent of the thiazolidinedione class, has been reported in vivo experiments to produce nearly 66% less vascular SMC proliferation; in the same study, troglitazone also inhibited PDGF-induced vascular SMC migration in vitro (87). The authors suggest that inhibition of tyrosine kinase-dependent growth factor action by troglitazone inhibits migration and proliferation of vascular SMCs. Another compound, SCH 13929 (2-bromomethyl-5-chlorobenzene sulfonylphthalimide), inhibits PDGF-induced migration and proliferation of SMCs by inhibiting binding of PDGF with its receptor owing to the interaction with the ligand (88). The potential of these compounds for prevention of restenosis remains to be proven.

**Drugs inhibiting intracellular (SMC) effects of PDGF.**

The antioxidant vitamin E has been reported to inhibit PDGF activity in vitro (89); these authors suggest that vitamin E may inhibit protein kinase C activity and, by preventing low density lipoprotein modification and uptake by macrophages, may inhibit the release of PDGF. Calcium antagonists have also been tried, because PDGF-induced intracellular calcium flux (resulting from stimulation of diglyceride formation) is considered an important component in postreceptor signaling pathways (90). A meta-analysis of five randomized trials involving 919 patients showed a reduction of 30% in the relative risk of developing restenosis after PTCA (91). Another trial, the Coronary Angioplasty Amlodipine in REStenosis (CAPARES) trial, has been designed to assess the influence of calcium antagonists on restenosis (92).

In a rat model, orally administered pemirolast or tranilast (antiallergic agents) started two days before arterial injury significantly reduced the extent of neointimal proliferation and intimal thickening (93); inhibition of activation of phospholipase C may be the mechanism of action, as phospholipase C is essential for cell proliferation stimulated by PDGF (94). In human trials, patients treated with pemirolast had significantly lower restenosis rates as compared with control subjects (95). Another compound demonstrated to have anti-PDGF activity is bromocriptine. In an in vitro study, bromocriptine significantly inhibited the proliferation of rat vascular SMCs (96). The inhibitory effect of bromocriptine on protein kinase C is postulated as a possible mode of anti-PDGF action, as protein kinase C is involved in PDGF-induced vascular SMC proliferation (97). The authors of that study also suggest that because bromocriptine is a serotonin antagonist, the antiproliferative effect could also result from the inhibition of stimulatory effect of serotonin on vascular SMCs. Rapamycin, an immunosuppressive agent of the macrolide group, has shown to exert an antiproliferative effect on rat aortic wall SMCs by inhibiting PDGF-induced vascular DNA synthesis—an effect postulated to occur by preventing cells from progressing from the G1 to S phase of the cell cycle (98).

**Other drugs that inhibit PDGF-induced SMC proliferation.**

Drugs that are not direct inhibitors of the PDGF pathway also inhibit PDGF-induced SMC proliferation. Prostaglandin \(E_1\), given as a continuous infusion after arterial injury in rats, significantly inhibited neointimal proliferation (99). Suppression of leukocyte activation (which can release PDGF) and increased intracellular cyclic adenosine monophosphate (which suppresses vascular SMC growth) are considered to be responsible for the effects of prostaglandin \(E_1\) on vascular SMCs (100). Finally, paclitaxel, a drug developed for therapy of breast and ovarian cancer, has also demonstrated an anti-PDGF effect in experimental studies. Paclitaxel induces the formation of abnormally stable and nonfunctional microtubules, leading to inhibition of cell replication (101). In a recent report, paclitaxel was shown to produce dose-dependent inhibition of PDGF-induced human arterial SMC migration and proliferation (102). In the same report, rabbits treated with local paclitaxel after balloon angioplasty showed significantly less neointimal proliferation and lower restenosis.

**Conclusions.** Of the three major factors contributing to restenosis after PTCA, elastic recoil and vessel wall remodeling have been addressed by the advent of stents. There is no effective therapy yet for the problem of neointimal proliferation, with the possible exception of brachytherapy. Platelets, by their capacity to adhere to the sites of arterial injury, to form aggregates and to secrete highly potent growth factors, the most important of which is PDGF, appear to play an important role in neointimal proliferation and development of restenosis. Many novel drugs and delivery systems targeted at platelets have shown success in experimental studies. However, the beneficial effects of the various drugs tested successfully in experimental studies have yet to be translated into a significant reduction in restenosis. The use of drug-encapsulated platelets and systemic or local delivery of anti-PDGF agents that inhibit the various levels of action of PDGF appear to hold promise in the prevention of restenosis.

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