Colchicine and Antineoplastic Therapy for the Prevention of Restenosis After Percutaneous Coronary Interventions

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The complexity of the events that culminate in intimal proliferation after arterial injury and similarities between this response and benign neoplasia suggest that conventional medical therapies will continue to be unsuccessful in preventing recurrent stenosis after percutaneous coronary revascularization. By preventing cell division after smooth muscle cell activation, antimitogenic therapy may inhibit the final common pathway in this complex chain of events and offset the apparent loss of local growth control. Colchicine, which causes metaphase arrest of cell division, has been shown in experimental studies to decrease the extent of atheromatous plaque formation and reduce the severity of arterial restenosis after balloon angioplasty. However, preliminary results from a randomized placebo-controlled clinical trial suggest that low dose colchicine (0.6 mg twice a day orally) does not prevent restenosis.

The use of more potent antineoplastic agents is limited by the potential for life-threatening side effects. It is possible that these adverse effects can be averted by using novel drug delivery systems to administer antimitogenic therapy locally at the site of arterial injury or by using low dose synergistic combinations of antiproliferative agents. This review examines the potential role of antimitogenic therapy in the prevention of restenosis after coronary interventions and considers the possibility of an overlap of the therapeutic realms of interventional cardiology and medical oncology.

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Despite intensive clinical and experimental investigation over the past decade, the incidence of recurrent stenosis after balloon dilation of coronary artery stenosis has remained remarkably constant. Although several animal studies of antiplatelet, anticoagulant and vasodilator therapies have reported favorable reductions in the severity of the intimal proliferative response to balloon injury, clinical studies (1-4) have failed to identify any single pharmacologic agent that consistently inhibits this response. More recently, attempts have been made to modify the endovascular injury inflicted during percutaneous revascularization procedures. These efforts have included the use of devices that excise, compress or heat atheromatous tissue and others that use laser energy to ablate obstructive material (5). Preliminary data from follow-up studies (6-8) suggest that these procedures may also be associated with an incidence of restenosis that is comparable with that of conventional balloon angioplasty. Moreover, histologic analyses (9,10) have suggested that the intimal proliferative response after these dissimilar injuries may be pathologically indistinguishable from the response to balloon dilation. These findings suggest a limited role for further modification in the nature of the arterial injury.

For this reason, although these new devices remain a valuable means of optimizing the initial procedural result (11), the focus of attention for many investigators has returned to the evaluation of pharmacologic preventive measures. The agents currently under experimental investigation include a variety of antineoplastic drugs. The use of such potent therapies has arisen from the recognition 1) of the complexity of the factors that lead to smooth muscle cell activation, and 2) of parallels between the smooth muscle cell proliferative response of restenosis and the apparently unrestricted growth of neoplastic cells.

Stimuli to Intimal Proliferation

The complexity of the vascular reparative response to injury has been well reviewed by several investigators (12-14). It now appears clear that multiple heterogeneous factors trigger a common response to injury (Fig. 1). This response consists of medial smooth muscle cell activation characterized by a change in smooth muscle cell phenotype from contractile to synthetic form, migration of both phenotypes from the arterial media to the intima, exuberant smooth muscle cell proliferation and subsequent synthesis and secretion of extracellular collagenous matrix (15-17). In some individuals, this process continues, apparently unpended by normal control mechanisms, until the neointima encroaches on the arterial lumen and compromises coronary
Figure 1. Stimuli to smooth muscle cell (SMC) proliferation after arterial wall injury. The vascular reparative response may be triggered by mechanical factors, elements of the coagulation system and the inflammatory response and changes in blood flow.

flow. The growth factors that trigger this response, including platelet-derived growth factor, fibroblast growth factor, epidermal growth factor, transforming growth factor α and insulin-like growth factor, are released from activated platelets, monocytes, endothelial cells and smooth muscle cells themselves. In addition, numerous other components released after arterial trauma, including endothelin (18), interleukin-1 (19) and leukotriene B₄ (20), have been shown to promote medial smooth muscle cell proliferation. Other factors, including stretch alone (21), may activate smooth muscle cells indirectly by inducing the formation of growth factor receptors (22) or directly by provoking the autocrine release of growth factors from smooth muscle cells themselves (23).

Whereas conventional medical approaches to the prevention of restenosis have attempted to inhibit individual components of this response, newer approaches may include inhibition of the induction and expression of smooth muscle cell growth factor receptors and interference with intracellular signal transduction. Less futuristic, perhaps, is the possibility that antimitogenic therapy may inhibit the final common pathway by preventing medial smooth muscle cell division and proliferation. Whether it is also necessary to prevent migration and collagen synthesis by nonproliferating smooth muscle cells is as present unknown.

Intimal Proliferation and Neoplasia

A second argument for the evaluation of antineoplastic therapy in this context is the analogy between intimal proliferation and benign neoplasia. In 1973, a pathologic study (24) of atherosclerotic lesions in black women who were heterozygous for glucose-6-phosphate dehydrogenase suggested that individual plaques were composed of cells that were monoclonal in origin. This raised the possibility that the underlying pathogenesis of atherosclerosis is focal neoplasia (24). Subsequent investigations (25) have been inconsistent in supporting or refuting this concept. An even closer analogy to the intimal proliferation of restenosis may be the formation of keloid scars from abnormal wound healing in genetically susceptible individuals. These benign dermal tumors have reduced growth factor requirements in culture and respond differently from unaffected skin to exogenous growth factors (26). These differences have been attributed to the autocrine production of growth factors by dermal cells, reduced production of growth-inhibitory peptides or alterations in growth factor receptor activity or postreceptor signal transduction (26).

It is clear from tritiated thymidine studies that experimentally induced intimal proliferative lesions and, presumably, the lesions of coronary restenosis after balloon dilation are not of monoclonal origin. The cells of these lesions may, however, have altered growth characteristics and responses to mitogens when compared with cells from primary atherosclerotic lesions. In a recent study by Dartsch et al. (27), cultured human smooth muscle cells obtained from atherectomy of recurrent femoral artery stenoses showed a significantly higher baseline growth rate and a heightened proliferative response to platelet-derived growth factor and a growth factor mixture isolated from bovine brain when compared with cells derived from advanced primary stenoses.

Perhaps one of the most intriguing similarities between restenosis and neoplasia is the apparent homology between platelet-derived growth factor and mitogens released from malignant neoplasms. A striking structural, functional and immunologic homology has been noted, for example, between platelet-derived growth factor and p28<sup>III</sup>, the transforming protein of the simian sarcoma virus (28), and a similar mitogen has been purified from human osteosarcoma cells. Human astrocytomas have also been shown to express both platelet-derived growth factor and its receptor genes, suggesting that platelet-derived growth factor may act as a potent protooncogene in the maintenance of growth of these tumors (29).

The analogy between coronary restenosis and focal benign smooth muscle cell neoplasia with failure of normal growth control mechanisms is, therefore, an attractive, if somewhat simplistic concept. Indeed, the term "malignant restenosis" has recently found its way into the lexicon of interventional cardiologists to describe the frustratingly rapid and intractable recurrence of coronary artery stenoses after a variety of percutaneous coronary interventions. With these considerations in mind, is there a role for antimitogenic therapy in the prevention of restenosis after balloon angioplasty? Two classes of antimitogenic agents (colchicine and the antineoplastic agents) warrant evaluation.

Colchicine

Mechanism of action. Colchicine is an alkaloid derived from the plant Colchicum autumnale. Its medicinal use was described as early as the 1st century AD and its principal mechanisms of action were described in 1889 by Pernice (30) and later evaluated in detail by Taylor (31). It binds with high
Methotrexate inhibits DNA synthesis (S phase) by preventing the formation of tetrahydrofolate. Affinity to tubulin, the subunit protein of microtubules, thereby inhibiting the assembly of these organelles and, to a variable extent, causing disassembly of microtubules that have already been formed. Because normal microtubule function is essential for spindle formation, colchicine therapy results in metaphase arrest of cell division (Fig. 2). In addition, colchicine inhibits other microtubule-dependent functions, including the secretion of polymorphonuclear cell and monocyte chemotactic factors (32,33), the synthesis and secretion of collagen (34) and lysosome degranulation after phagocytosis (30). Furthermore, colchicine therapy has been shown to increase collagenase activity (35) and inhibit platelet aggregation and secretion (36). Its principal therapeutic use has been in the treatment of acute gouty arthritis and as prophylactic maintenance therapy for hyperuricemia.

The anti-inflammatory and antifibrotic activity of the drug have prompted its evaluation in several nonrheumatologic conditions. In a recent randomized clinical trial of patients with hepatic cirrhosis (37), colchicine therapy was associated with a reduction in the severity and extent of perportal inflammation and fibrosis and an increase in long-term survival. Colchicine has also been used as an antineoplastic agent for malignant conditions, including acute leukemia. Its use for these conditions has been limited, however, by high incidence of side effects, including hemorrhagic gastroenteritis and bone marrow depression. Nonetheless, its broad spectrum of antiproliferative, anti-inflammatory and antifibrotic activity suggests a potential role for colchicine in the prevention of restenosis after percutaneous coronary revascularization procedures.

**Experimental studies of colchicine and vascular injury.** This possibility is supported by a small number of experimental studies. Colchicine has been shown to inhibit to a variable extent the development of atherosclerotic plaques in several animal models (38-40). Hollander et al. (38), for example, showed a reduction in the severity of visible aortic atherosclerosis in rabbits fed an atherogenic diet. The lesions that developed in the treated animals contained less free and ester cholesterol and less collagen and elastin than did the lesions of rabbits fed an atherogenic diet alone. Similarly, in rabbits with established atherosclerosis (39), colchicine therapy during a subsequent hypocholesterolemic regression diet appeared to reduce the severity of intimal fibrous tissue formation. Only one study (41) reported the use of colchicine to prevent intimal thickening after balloon arterial injury. In the atherosclerotic rabbit model, Currier et al. (41) showed a moderate reduction in the severity of restenosis 4 weeks after iliac arterial balloon dilation in animals treated with high dose (0.2 mg/kg per day) but not low dose (0.02 mg/kg per day) colchicine (Fig. 3). It should be noted, however, that the chronic high dose colchicine therapy used in this study would almost certainly not be tolerated in clinical studies.

**Clinical restenosis trials.** Low dose colchicine therapy was recently evaluated in a randomized placebo-controlled clinical trial at the Mid-America Heart Institute (42). The majority of the 197 patients enrolled in the study had multileision or multivessel disease and approximately 500 coronary stenoses were dilated. Patients were randomly assigned to receive colchicine (0.6 mg twice a day orally) or placebo commencing on the day before the angioplasty procedure and continuing throughout the study period. Drug efficacy was evaluated with use of exercise thallium scintigraphy 3 months after the procedure and repeat coronary angiography at 6 months. Although this low dose therapy was reasonably well tolerated in the majority of patients, adverse effects requiring termination of drug therapy occurred in approximately 7% of the treated patients. In a preliminary analysis of the data from the first 145 patients completing the study, the incidence of restenosis at 6 months was 46% in the colchicine-treated group compared with 47% in the placebo group (p = NS).

**Antineoplastic Therapy**

**Experimental studies.** The use of more potent antimitogenic agents for the prevention of restenosis has not been well studied, predominantly because of the potential hazards of such toxic therapy and the relatively benign clinical consequences of restenosis. The agents used most frequently to treat malignancies of mesenchymal cell origin include vincristine, actinomycin D, doxorubicin, cyclophos-
phamide and methotrexate. A regimen of combined low dose intravenous vincristine (0.075 mg/kg) and actinomycin D (0.015 mg/kg) was recently evaluated in a rabbit model of aortic endothelial injury (43). Three days after endothelial denudation, the animals treated with antineoplastic therapy had fewer subintimal smooth muscle cells (155% versus 223% of the control number) and less evidence of activation (nuclear euchromatization and endoplasmic reticular content) by electron microscopy when compared with the control untreated animals. Whether this therapy has any impact on the degree of subsequent intimal thickening in this model is unknown because longer-term studies were not reported.

Local drug delivery at site of angioplasty. A more acceptable approach to the systemic administration of potentially life-threatening drug therapy to patients with nonmalignant conditions may be local drug delivery at the site of balloon angioplasty. We investigated the use of the antimetabolite methotrexate given locally for the prevention of intimal proliferation after balloon arterial injury in the porcine carotid artery model (44). Methotrexate is a competitive inhibitor of dihydrofolate reductase, the enzyme responsible for the synthesis of tetrahydrofolate from dihydrofolate. Inhibition of the enzyme results in a cellular deficiency of tetrahydrofolate, a compound essential for the synthesis of purines and pyrimidines and thus for the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Cellular proliferation is therefore prevented by inhibition of the S phase of the cell cycle (Fig. 2). Unlike most antineoplastic agents, the effects of methotrexate can be rapidly reversed with high dose folinic acid (5-formyl tetrahydrofolate) rescue. For this study, the drug was delivered through a specially designed infusion catheter. The catheter has a 4.3F triple lumen shaft with a distal balloon made of polyethylene terephthalate. The balloon has 28 holes, 25 μm in diameter, in longitudinal rows (Fig. 4). In a previous study (45) evaluating this balloon, fluoresceinated heparin delivered at 5 atm pressure for 1 min penetrated the full thickness of a canine arterial wall and remained detectable for 48 to 72 h.

After balloon angioplasty, bilateral carotid angioplasty was performed by direct cutdown under general anesthesia in 15 Yucatan minipigs (46). In 10 of these animals, methotrexate (6.25 mg/ml) was then infused locally at the site of balloon dilation in one carotid artery. The contralateral side was infused with 0.9% saline solution. The local administration of methotrexate did, in fact, result in transient low therapeutic systemic drug levels ranging from 0.7 to 22 μg/liter (therapeutic range 10 to 1,000). Because the contralateral side was exposed, if only transiently, to circulating methotrexate, five additional animals underwent carotid angioplasty and bilateral infusion of saline solution as "true" control animals. Tritium-labeled methotrexate was used to determine the duration of local drug effect. The intramural drug concentration in the treated carotid artery at the site of balloon injury was 1,000-fold greater than the serum concentration 2 h after drug administration and very high tissue concentrations were maintained for ≥7 days. After the surgical procedure, each animal was maintained on a normal diet for 4 weeks, at which time it was killed and the carotid arteries were resected for histologic analysis.

Preliminary data from this study (46) suggest that the intimal proliferative response is not abolished and may not even be attenuated using this dose of methotrexate and this delivery system. Whether the drug itself is ineffective or the duration of its activity is inadequate with this approach is unclear at present and is the subject of ongoing investigation.

Other approaches. The potential role of long-term local drug delivery at the site of arterial dilation is the focus of intense research in several academic centers. Other drug delivery systems that may prove to be valuable include drug-implregnated polymer-coated metallic stents, biodegradable drug-eluting polymer stents (47) and genetically primed endothelial cells to coat metallic stents (48) or be delivered directly as a local endothelial cell covering (49). These systems may allow the safe use of potent antiproliferative therapies, including antimitogenic agents, in high local concentrations without systemic side effects.

Several other approaches warrant further investigation. It may be possible, for example, to find synergistic drug combinations to inhibit intimal proliferation. Low dose antimitogenic therapy given orally or parenterally for a period of 7 to 10 days may be adequate well tolerated therapy if combined with a potent growth factor antagonist. Alternatively, because a substantial proportion of the neointima is composed of collagenous matrix (some of which may be derived from nonproliferating cells), the combination of an antineoplastic agent with a powerful inhibitor of collagen formation may be valuable.

Conclusions

On purely theoretic grounds, antimitogenic therapy appears to offer some potential as an approach to the prevention of restenosis. Its use may be limited, however, not only by troublesome systemic side effects, but also by the potentially deleterious effects of inhibition of regrowth of the endothelium. Conceivably, the latter effect may actually
increase the severity of restenosis by prolonging the period of exposure of subintimal tissue. If these therapies do prove to be successful in reducing the incidence of restenosis, consideration will need to be given to patient selection criteria for treatment because approximately two thirds of patients undergoing their first percutaneous revascularization procedure do not develop recurrent stenosis. One possible means of identifying individuals at particularly high risk of restenosis may be the examination of material obtained by coronary angiectomy (9, 10, 27). In much the same way as biopsy of a neoplastic growth can provide information regarding its activity and growth potential, endovascular biopsy using an angiectomy device may give useful information about the probability of subsequent restenosis. This possibility is the subject of ongoing research at our institution.

From what is known about the pathologic response to balloon angioplasty and related interventions, it seems likely that a combined mechanical and pharmacologic approach will be necessary to optimally treat coronary stenosis and prevent subsequent restenosis. Whether the pharmacologic component of that therapy should be drugs as potentially toxic as the antineoplastic agents is at present unclear. Further evaluation of these agents in experimental models of restenosis seems warranted, however, because this may at the very least provide valuable insights into the pathogenesis of this highly complex process.

References