Editorial

Therapeutic approaches to the control of fibrocellular intimal hyperplasia after angioplasty

Enthusiasm for the technique of percutaneous transluminal coronary angioplasty has been tempered by the realisation that 25–50% of patients have a recurrence of their symptoms in 3–6 months because of restenosis at the site of the original atheromatous lesion.¹ This is due not to local thrombosis or to the reaccumulation of atheroma but to a rapid growth of vascular smooth muscle cells, both within the intima and migrating from the media, which produces a new lesion known as fibrocellular intimal hyperplasia (FCIH).² There is probably some degree of FCIH in all patients after angioplasty; and its rate of development and final quantity determine the recurrence of angina. FCIH is not unique to the vessel injured by a balloon catheter: intimal hyperplasia is a feature of the “onion skin” lesion of malignant hypertension and systemic sclerosis, the plexiform lesion in the pulmonary arterioles in pulmonary hypertension, and late vein graft occlusion.³ More recently intimal hyperplasia has been identified as the cause of the diffuse vascular disease which occurs after cardiac transplantation⁴ and it is the main cause of graft failure after 5 years. The vessel wall seems to have a single response to various insults on the endothelium, media, and adventitia. These insults include chronic immunological injury, sustained pressure increases, rheological changes, or direct physical distension.

Animal models

There has been considerable debate about the relevance of established animal models of FCIH to the clinical problems, particularly to restenosis after angioplasty.⁵ In some species—for example, the rat—the normal intima consists of only a layer of endothelium and so the vascular smooth muscle cells in FCIH must be derived from the media, while in others, such as humans and the pig, the intima is much more substantial and does contain vascular smooth muscle cells. The contribution of the intimal vascular smooth muscle cells and of cells such as macrophages within the atheromatous plaque to the subsequent development of FCIH is not known. Atheroma has a complex pathology and is many years in the making. The best models of its genesis are expensive and have given us only limited insight.⁶ FCIH on the other hand seems to develop relatively rapidly, particularly after angioplasty when it is established within weeks or a few months. Animal models of FCIH can be easily induced by vascular distension with a Fogarty balloon catheter or angioplasty catheter. The histological appearance of FCIH varies somewhat between species but the accumulation of vascular smooth muscle cells, and extracellular matrix is characteristic of the lesion.⁵ Smooth muscle cells within the lesion have a different phenotype from that of quiescent medial vascular smooth muscle

cells, there being a change in the contractile protein isoforms, increased cellular synthetic apparatus, and increased motility of cultured cells derived from the lesion.^{6–8} Synthesis of matrix proteins, such as tenascin, is increased⁹ and tissue type plasminogen activator and urokinase are expressed by the smooth muscle cells of the injured vessel.¹⁰ Endothelial regrowth may be incomplete though the luminal smooth muscle cells become flattened and superficially are similar in appearance to the endothelium.^{7,11} There is evidence also of persistent impairment of endothelial cell function.¹²

Clinical trials

The development of restenosis at the lesion site in patients undergoing angioplasty has been markedly resistant to empirical drug treatment: corticosteroids, aspirin, fish oils, calcium channel blockers, and angiotensin converting enzyme inhibitors have all been tried without success.¹³ The results of studies of the effects of hirudin or self-administered subcutaneous heparin are awaited and a further trial is planned to investigate the therapeutic potential of monoclonal antibodies to the platelet adhesion molecule GPIIb/IIIa.

Pathogenesis

Examination of the nature of the injury produced by the balloon dilatation gives some insight into the pathogenesis of the restenotic lesion. The atheroma is compressed into the wall of the artery but the process, by necessity, also destroys or damages the overlying endothelium and stretches the media and adventitia. Some degree of dissection is probably inevitable.² There is evidence from both experimental models of angioplasty¹⁴ and from clinical audit¹⁵ that the development of FCIH is related to the amount of distension of the vessel by the balloon. In the clinical situation this is difficult to control precisely because the relief of the patient's symptoms requires that the lumen size is increased and displacement of the atheroma adds to the direct pressure from the balloon catheter.

Much of the research into the pathogenesis of FCIH after balloon catheter injury has focused on the role of various autocrine and paracrine growth factors and cytokines. As might be expected with an injury that causes stripping of the endothelium and direct physical damage to medial vascular smooth muscle cells and adventitial nerves and vasa vasorum, the mediator involvement is complex. Platelet derived growth factor (PDGF),¹⁶ basic fibroblast growth factor (bFGF),¹⁷ transforming growth factor beta (TGF- β)¹⁸ and interleukin-1- α ¹⁹ are among the many factors that have been implicated in the

Segments of rat carotid artery after balloon angioplasty. The control segment was not locally treated. Inactive oligonucleotide was applied to the sense *c-myb* segment. Carrier gel for the oligonucleotides was applied alone to the pluronic gel segment. All these three controls show considerable intimal thickening encroaching on the lumen. In the segment to which antisense oligonucleotide was applied binding of mRNA for *c-myb* has suppressed smooth muscle proliferation and intimal thickening.

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pathogenesis of FCIH. Clearly the response to the balloon catheter injury of the vessel is so fundamental that complex interacting cascades of growth factors ensure the development of FCIH and it is unlikely that a single pivotal factor amenable to pharmacological intervention will be easily identified. In the rat model of FCIH high doses of angiotensin converting enzyme inhibitors substantially inhibit intimal thickening after balloon catheter injury of the rat carotid artery²⁰ but clinically relevant doses of ACE inhibitors did not affect the incidence of restenosis after coronary angioplasty.²¹ It may be that the stimulatory cascades of growth factors and cytokines in humans and in the rat have a different balance that influences the response of the lesion to drugs.

New approaches to prevention

Nevertheless, FCIH develops because of proliferation and migration of vascular smooth muscle cells. However, this response is likely to involve mechanisms fundamental to growth and repair and control of FCIH will probably necessitate the local application of agents that will inhibit either or both proliferation and migration. These may be pharmacological, physical (for example radiation) or genetic. Transfection of cells for subsequent transplantation and direct transfection into the arterial wall of marker genes such as β -galactosidase,^{22–24} luciferase,²⁵ and human adenosine deaminase²⁶ and genes of proteins with therapeutic potential such as tissue plasminogen activator²⁷ has already been demonstrated. Simons *et al* successfully inhibited FCIH induced by Fogarty balloon catheter dilatation of the rat carotid by the application of a phosphothiolated antisense *c-myb* oligonucleotide to the adventitial surface of the injured vessel (figure).²⁸ The proto-oncogenes such as *c-fos*, *c-myc* and *c-myb* are universally expressed during cell division and may be induced by a wide range of stimuli including physical stresses.^{29–30–32} Antisense oligonucleotides are short lengths of single stranded DNA that are complementary (antisense) to lengths of DNA in part of the target gene or to the RNA transcript from it. The oligonucleotides inhibit the function of the gene by binding to these sequences.³³ Chemical modification of the oligonucleo-

tides confers resistance to degradation by nucleases and improves stability.³⁴ Targeting the early cellular responses to injury may minimise any possible inter-species differences in the pathogenesis of the lesion but genes expressed uniquely by proliferating or migrating vascular smooth muscle cells may be more specific targets.

Simons *et al*²⁸ delivered their synthetic antisense oligonucleotides to the adventitial surface of the injured vessel and clearly this would not be feasible for routine clinical use. However, administration of the therapeutic agents directly to the vessel wall requires only minor modification of catheter hardware already available. Such modifications include double balloons to isolate the segment of artery into which the therapeutic agent is infused via a central pore,^{22–24} microperforation of the balloon to allow instillation of agents into the wall, and perfusion systems to permit distal blood flow during the procedure²⁵ (though instillation of the transfection vector or the oligonucleotide may take only a few minutes). The duration of activity of the modified antisense oligonucleotides or of the duration of expression of transfected genes of proposed therapeutic function, and the longer term effects of retroviral transfection vectors will have to be ascertained before such agents can be applied to humans. Animal models of FCIH will have a pivotal role in these studies and costing, handling, and housing advantages make the rat the natural choice for primary studies though confirmatory studies in pigs or primates may follow. It can be envisaged that in the future percutaneous transluminal coronary angioplasty will be coupled to the local application of a therapeutic agent aimed at inhibiting the development of FCIH and resulting restenosis.

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- 1 Greuntzig AR, King SB, Schlumpf M, Seigenthaler N. Long term follow up after percutaneous coronary angioplasty. The early Zurich experience. *N Engl J Med* 1987;316:1127–32.
- 2 Waller BF, Orr CM, Pinkerton CA, VanTassel JW, Pinto RP. Morphological observations late after balloon angioplasty: mechanisms of acute injury and relationship to restenosis. *Radiology* 1990;174: 961–7.

- 3 Dilley RJ, McGeachie JK, Prendergast FJ. A review of the histological changes in vein to artery grafts, with particular reference to intimal hyperplasia. *Arch Surg* 1988;123:691-6.
- 4 Billingham ME. The post-surgical heart—the pathology of cardiac transplantation. *Am J Cardiovasc Pathol* 1988;1:319-34.
- 5 Muller DWM, Ellis SG, Topol EJ. Experimental models of coronary artery restenosis. *J Am Coll Cardiol* 1992;19:418-32.
- 6 Bauriedel G, Windstetter U, DeMaio SJ, Kandolf R, Hofling B. Migratory activity of human smooth muscle cells cultivated from coronary and peripheral primary and restenotic lesions removed by percutaneous atherectomy. *Circulation* 1992;85:554-64.
- 7 Kocher O, Gabbiani F, Gabbiani G, et al. Phenotypic features of smooth muscle cells during evolution of experimental carotid artery intimal thickening. *Lab Invest* 1991;65:459-70.
- 8 Leclerc G, Isner JM, Kearney M, et al. Evidence implicating non muscle myosin in restenosis. *Circulation* 1992;85:543-53.
- 9 Hedin U, Holm J, Hansson GK. Induction of tenascin in rat arterial injury. *Am J Pathol* 1991;139:649-56.
- 10 Clowes AW, Clowes MM, Ay YP, Reidy MA, Belin D. Smooth muscle cells express urokinase during mitogenesis and tissue type plasminogen activator during migration in injured rat carotid artery. *Circ Res* 1990;67:61-7.
- 11 Lindner V, Reidy MA, Fingerle J. Regrowth of arterial endothelium. Denudation with minimal trauma leads to complete regrowth. *Lab Invest* 1989;61:556-63.
- 12 Shimokawa H, Aarhus LL, Vanhoutte PM. Porcine coronary arteries with regenerated endothelium have a reduced endothelium dependent responsiveness to aggregating platelets and serotonin. *Circ Res* 1987;61:256-70.
- 13 Brady A, Warren J. Angioplasty and restenosis. *BMJ* 1991;303:729-30.
- 14 Capron L, Bruneval P. Influence of applied stress on mitotic response of arteries to injury with a balloon catheter: quantitative study on rat thoracic aorta. *Cardiovasc Res* 1989;23:941-8.
- 15 Beatt KJ, Serruys PW, Luijten HE, et al. Restenosis after coronary angioplasty: the paradox of increased lumen diameter and restenosis. *J Am Coll Cardiol* 1992;19:258-66.
- 16 Jawein A, Bowen-Pope DF, Lindner V, Schwartz SM, Clowes AW. Platelet derived growth factor promotes smooth muscle cell migration and intimal thickening in a rat model of balloon angioplasty. *J Clin Invest* 1992;89:507-11.
- 17 Lindner V, Lappi DA, Baird A, Majack RA, Reidy MA. Role of basic fibroblast growth factor in vascular lesion formation. *Circ Res* 1991;68:106-13.
- 18 Majesky MW, Lindner V, Twardzik DR, Schwartz SM, Reidy MA. Production of transforming growth factor β during repair of arterial injury. *J Clin Invest* 1991;88:904-10.
- 19 Brody JJ, Pickering NJ, Capuzzi DM, Fink GB, Can CA, Gomez F. Interleukin-1 alpha as a factor in occlusive vascular disease. *Am J Clin Pathol* 1992;97:8-13.
- 20 Powell JS, Clozel JP, Muller RK, et al. Inhibitors of angiotensin converting enzyme prevent myointimal proliferation after vascular injury. *Science* 1989;245:186-8.
- 21 Mercator study group. Does the new converting enzyme inhibitor cilazapril prevent restenosis after percutaneous transluminal angioplasty. *Circulation* 1992;86:100-10.
- 22 Nabel EG, Plautz G, Boyce FM, Stanley JC, Nabel GJ. Recombinant gene expression in vivo within endothelial cells of the arterial wall. *Science* 1989;244:1342-4.
- 23 Nabel EG, Plautz G, Nabel GJ. Site specific gene expression in vivo by direct gene transfer into the vessel wall. *Science* 1990;249:1285-8.
- 24 Plautz G, Nabel EG, Nabel GJ. Introduction of vascular smooth cells expressing recombinant genes in vivo. *Circulation* 1991;83:578-83.
- 25 Chapman GD, Lim CS, Gammon RS, et al. Gene transfer into coronary arteries of intact animals with a percutaneous balloon catheter. *Circ Res* 1992;71:27-33.
- 26 Lynch CM, Clowes MM, Osborne WRA, Clowes AW, Miller AD. Long term expression of human adenosine deaminase in vascular smooth muscle cells of rats: a model for gene therapy. *Proc Natl Acad Sci USA* 1992;89:1138-42.
- 27 Dichck DA, Neville RF, Zweibel JA, Freeman SM, Leon MB, Anderson FW. Seeding of intravascular stents with genetically engineered cells. *Circulation* 1989;80:1347-53.
- 28 Simons M, Edelman ER, DeKeyser J-L, Langer R, Rosenberg RD. Antisense c-myc oligonucleotides inhibit intimal arterial smooth muscle cell accumulation in vivo. *Nature* 1992;359:67-70.
- 29 Evans GI. Introduction: the great nuclear debate. *Seminars in Cancer Biology* 1990;1:351-8.
- 30 Izumo S, Nadel-Ginard B, Mahdavi V. Proto-oncogene expression and reprogramming of cardiac gene expression produced by pressure overload. *Proc Natl Acad Sci USA* 1988;85:339-43.
- 31 Bennett MR, Evan GI, Newby AC. Central role of the c-myc proto-oncogene in vascular smooth muscle cell proliferation and cell death [abstract]. *Br Heart J* 1993;69(Suppl):P57.
- 32 Anglin S, Bennett MR, Jagoe R, Evan G, McEwan JR. An antisense oligonucleotide to c-myc inhibits fibrocellular intimal hyperplasia in a rat carotid model of angioplasty [abstract]. *Br Heart J* 1993;69(Suppl):P88.
- 33 Akhtar S, Juliano RL. Antisense DNA oligonucleotides as potential therapeutic agents. *Pharm J* 1991;247:89-92.
- 34 Agrawal S, Temsamani J, Tang JY. Pharmacokinetics, biodistribution, and stability of oligonucleotide phosphorothiolates in mice. *Proc Natl Acad USA* 1991;88:7595-9.