

REVIEW

Drug eluting stents: are human and animal studies comparable?

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Animal models of stenting probably predict human responses as the stages of healing are remarkably similar. What is characteristically different is the temporal response to healing, which is substantially prolonged in humans. The prevention of restenosis in recent clinical trials of drug eluting stents may represent a near absent or incomplete phase of intimal healing. Continued long term follow up of patients with drug eluting stents for major adverse cardiac events and angiographic restenosis is therefore imperative.

treatments for human use. It can be argued that insufficient preclinical testing may have led to the recent failures of actinomycin-D (European Society of Cardiology Congress 2002, unpublished data) and the paclitaxel derivate (QP2 or 7-hexanoyltaxol) eluting polymer stents⁹ in de novo and restenotic lesions. It should be recognised that the vast knowledge of vascular healing and repair derived from animal studies is echoed in today's clinical achievements in the field of stent restenosis.

COMPARISON OF VASCULAR HEALING IN ANIMALS AND HUMANS

In animals or humans, the local response to a bare stainless steel stent in normal or diseased atherosclerotic coronary arteries follows a distinct pattern of arterial injury and repair accompanied by some degree of neointimal formation and endothelialisation. These healing events, however, can be notably altered with the addition of polymers, antirestenotic drugs, or both. Nevertheless, it is poorly appreciated that neointimal responses are exaggerated and that the time course of healing is more prolonged in humans than in animals.^{10,11} A comparison of arterial healing after coronary stenting in animals and humans is shown in fig 1. In a morphometric analysis of over 40 human stents collected at necropsy, peak neointimal thickness (mean (SD), 0.78 (0.37) mm) occurs between six months and one year, with approximately 22% regression in neointimal growth after one year. In contrast, neointimal formation in stented pig coronary arteries is maximal at one month (0.33 (0.24) mm), with around 25% lesion regression taking place over the following three to six months. Thus the response to healing after placement of a bare stainless steel stent in a human coronary artery is five to six times longer than in pig or rabbits. This concept is essential for the evaluation of a drug eluting stent: the interval from implantation to the actual data collection becomes crucial to the final outcome of testing. For a better understanding of the differences in the stent healing, a brief review of stent pathology in human coronary arteries and animals is useful to highlight temporal differences in vascular healing.

Over recent decades, percutaneous interventions have emerged as the preferred treatment for coronary artery disease. In the USA alone, more than one million percutaneous interventions are undertaken annually and more than 80% involve the use of coronary stents.¹ Although coronary stenting has had a dramatic impact on restenosis rates, restenosis still occurs in up to 30% of cases.² Moreover, the treatment of in-stent restenosis is a greater technical challenge than restenosis following balloon angioplasty alone, and often leads to additional catheter based or surgical revascularisation attempts. Drug eluting stents have emerged recently as promising agents in the prevention and treatment of stent related restenoses. Both sirolimus eluting and paclitaxel eluting stents represent an exciting breakthrough treatment, with remarkably low coronary restenosis rates of 0% to 9% at six and 12 months (unpublished data, ELUTES, ASPECT, TAXUS I, and SIRIUS trials, 2002).³⁻⁶ These impressive results have led some clinicians to speculate that drug eluting stents have remedied the problem of in-stent restenosis.

In this atmosphere of heightened enthusiasm, however, still linger the preclinical animal studies of sirolimus eluting (Carter AJ, 2002; unpublished results) and paclitaxel eluting stents (Heldman AW, 2002; unpublished results),⁷ showing efficacy at one month with lack of benefit by three and six months. The disparate results between six month clinical success and the failure of long term efficacy in animals has generated a scepticism that animal models do not accurately reflect the response to drug eluting coronary stents in humans.⁸ Although this tenet continues to gain momentum, is the complete dismissal of preclinical animal work justified? This fundamental question is essential, as pre-clinical testing is ingrained in the regulatory approval process traditionally used to determine the safety and potential efficacy or equivalency of

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Abbreviations: ASPECT, Asian paclitaxel eluting stent clinical trial; ELUTES, evaluation of paclitaxel-eluting stent; IVUS, intravascular ultrasound; MACE, major adverse cardiac event; RAVEL, randomised study with the sirolimus eluting velocity balloon expandable stent in treatment of de novo native coronary artery lesions; SIRIUS, sirolimus eluting stent

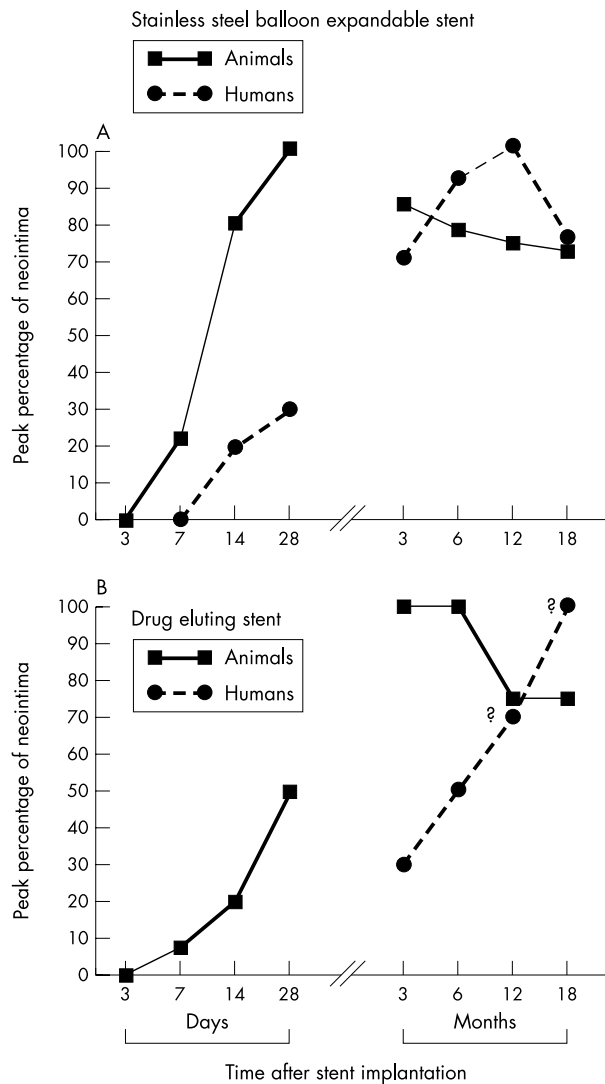


Figure 1 Line plot showing the temporal relation of peak neointimal growth in animals and humans following the placement of either “bare” stainless steel (A) or a drug eluting sirolimus or paclitaxel stent (B). The plots are predominantly derived from morphometric analysis of pig and human coronary stents; the drug eluting stent data in humans are projected from angiographic results.⁵ In animals, peak neointimal growth in stainless steel stents is observed at 28 days, compared with 6–12 months in humans. In human coronary arteries with drug eluting stents, the precise time course of peak neointimal growth is unknown (?). Animal studies of drug eluting stents, however, show favourable results at 28 days, with a lack of sustained efficacy at 3 and 9 months. The generalised delayed healing with drug eluting stents is thought to occur secondarily from an inhibition of smooth muscle cell proliferation and migration, and/or from the suppression of inflammation.

STENT HEALING

Animal models

The results of animal model studies are illustrated in figs 1 and 2. Although most tests of stent efficacy are performed at 28 days, there are a surprisingly few long term morphological studies on restenosis in pig coronary arteries.^{12–15} The early (one to three days) morphological response to stenting predominantly consists of platelet/fibrin deposition surrounding struts and scattered neutrophils within adherent luminal thrombi. By day 7, organising mural thrombi extending between stent struts contain smooth muscle cells and macrophages with scattered lymphocytes, red cells, and luminal endothelial cells. At 14 days, fibrin is still present with a few chronic inflammatory cells remaining around stent struts. At

this stage, the neointima contains few smooth muscle cells within a proteoglycan-rich matrix. By 28 days, the neointima contains a larger number of smooth muscle cells, proteoglycans, and type III collagen, with rare macrophages and giant cells around stent struts; fibrin is usually absent. The rate of neointimal expansion is greatest between 7–14 days, with maximal thickness achieved at one month. Over the next three to six months, the extracellular matrix becomes enriched in collagen type I, with neointimal shrinkage and remodelling. Cell proliferation in the neointima peaks at seven days, is reduced by approximately half at 14 days, and returns to a low baseline level by one month. Of note, stented rabbit iliac arteries follow a time course of healing similar to pig coronary arteries.

Human studies

The results of human studies are illustrated in figs 1 and 3. In stented human coronary arteries, platelet and fibrin deposition persists up to 14 and 30 days, respectively.¹⁰ Inflammatory cells, consisting of polymorphonuclear leucocytes and macrophages, are present by one to three days, and macrophages persist for at least three months. T lymphocytes appear at two to three weeks and persist beyond six months. Collections of smooth muscle cells—the main cellular component of the restenotic lesion—are evident by 14 days following stenting. The extracellular matrix, composed initially of proteoglycans and type III collagen, is gradually replaced by type I collagen past 12 months. The time course of intimal smooth muscle cell proliferation in relation to in-stent restenosis in humans is not known. Cell proliferation studies in human restenotic coronary atherectomy tissue retrieved from a few days to just beyond one year have thus far generally shown a low proliferation index without the characteristic peak found in existing animal models of angioplasty and stenting.¹⁴ Clearly, significantly more rapid proliferative events appear to occur in animals as distinct from human restenotic coronary arteries. Furthermore, rather than a simple proliferative response, smooth muscle cell migration from within the plaque or media to the expanding neointima may be the more dominant factor contributing to in-stent restenosis in humans.

TEMPORAL DIFFERENCES IN ARTERIAL HEALING IN HUMANS AND ANIMALS

One obvious explanation for the delayed arterial healing in humans is the underlying atherosclerotic process, which usually manifests in the fifth to sixth decade of life. Arterial interventions in animals are usually performed in young adults, and stents are typically placed in apposition to a normal smooth muscle-rich medial wall without inflammation. The absence of atherosclerotic disease is likely to contribute to a more predictable healing response in animals. In contrast, in diseased human coronary arteries, at least 70% of the stent is in direct contact with the underlying atherosclerotic plaque.^{10–11} The physical components of the lesion relative to the position of the stent probably affect the local response to healing. For example, stent struts in proximity to a necrotic core are exposed to few smooth muscle cells and thus heal slower than stents in direct contact with areas of adaptive intimal thickening, which contain an abundance of smooth muscle cells.¹⁰ Similarly, stents overlying calcified and densely fibrotic plaques also take longer to develop a neointima, as these plaques are also relatively hypocellular and must recruit smooth muscle cells from other remote areas of the arterial wall to cover bare struts.

The differential rate of healing between animals and humans may also be proportional to the longevity of the species. The typical life span of a human is more than 70 years; in contrast, pigs have a life span of 16 years, and rabbits 5–6 years. The age dependent biological differences in healing rates are exemplified in animal models of cutaneous wounds.

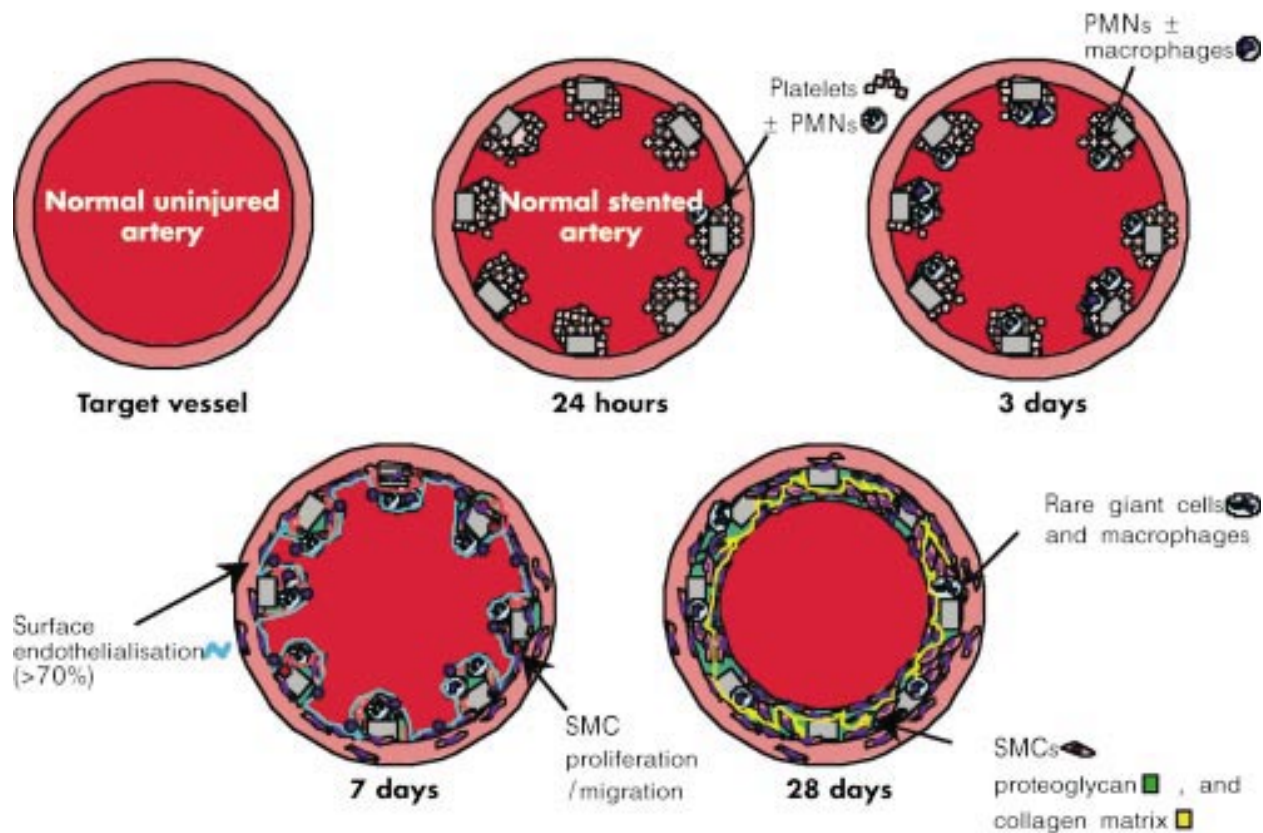


Figure 2 Illustration of the vascular response to a balloon expandable stainless steel stent implanted in a normal pig coronary or rabbit iliac artery. PMNs, polymorphonuclear leucocytes; SMCs, smooth muscle cells.

This analogy may be appropriate to in-stent restenosis, as the developing neointima is similarly considered to be a response to traumatic injury. In the pig, the extent of cutaneous re-epithelialisation declines with age, partly because of a decrease in the expression of growth factors.¹⁵ Further, wound contraction “remodelling” is accelerated in juvenile pigs. The type of injury is another consideration; wound healing is delayed in traumatic compared with surgically induced injury, and if the injury site is large.^{16,17} Human coronary stenting is often associated with extensive local trauma characterised by plaque splitting and medial disruption. Conversely, most stents in animals are deployed in normal arteries with 1:1.1 stent to artery ratio, resulting in only mild arterial injury.¹⁸

LESSONS LEARNED FROM BRACHYTHERAPY

Brachytherapy in animal models—catheter (β or γ radiation) or stent (β radiation) based—inhibits neointimal formation, with evidence of incomplete healing at one to three months.^{19–21} Continued healing, however, is accompanied by neointimal growth, and six month brachytherapy studies in animals fail to show a benefit. For example, Coussement and colleagues, using ¹⁸⁶Re β radiation (20 Gy dose) delivered through a balloon at six months in pig balloon injured coronary arteries, showed a significant decrease in lumen size with a reciprocally greater neointimal area than control non-radiated balloon injured arteries.²¹ Persistent fibrin deposition within the neointima was a notable finding in the radiated arteries. Complete endothelialisation was absent, a potential mechanism of late subacute thrombosis in animals and humans.^{21–24}

The lack of sustained efficacy after brachytherapy in animals stands in direct contrast to early clinical trials, in which reduced arterial stenosis was evident at six months. A likely explanation

is that healing occurs more rapidly in normal animal arteries because eventually there is a progressive arterial stenosis between six months and three years, as reported in patients receiving brachytherapy.⁸ The longest term human coronary brachytherapy data available (five years) show a mean (SD) arterial stenosis of 50.5 (22.9)% (range 19.4–100%) accompanied by positive remodelling, excessive adventitial fibrosis, and intimal calcification (Waksman R, 2001; unpublished data). Lumen loss and neointimal growth are more dramatic in radioactive stents analysed between six months and one year.²⁵ Taken together, these findings lend strong supportive evidence of late lumen loss in radiated arteries in humans and closely parallel the negative results in animal studies. The pathology of delayed healing with radiation is not unlike that of drug eluting stents showing persistent intimal fibrin deposition, inflammation, a paucity of smooth muscle cells in a proteoglycan-rich matrix, and incomplete endothelialisation. The similarity in histology raises the possibility that, like brachytherapy, neointimal growth with drug eluting stents will be only delayed rather than prevented.

HOW DO WE INTERPRET THE RESULTS OF DRUG ELUTING STENTS IN ANIMALS?

Sirolimus eluting or paclitaxel eluting stents at one month in pig and rabbit arteries show delayed healing, characterised by persistent fibrin deposition, variable inflammation, and incomplete endothelialisation. Those drugs that cause medial necrosis and positive remodelling (for example, actinomycin-D or paclitaxel) may induce exuberant neointimal formation at sites adjacent to the normal arterial wall (Virmani R, 2002; unpublished observations). The histological findings of drug eluting stents at one month in pig coronary

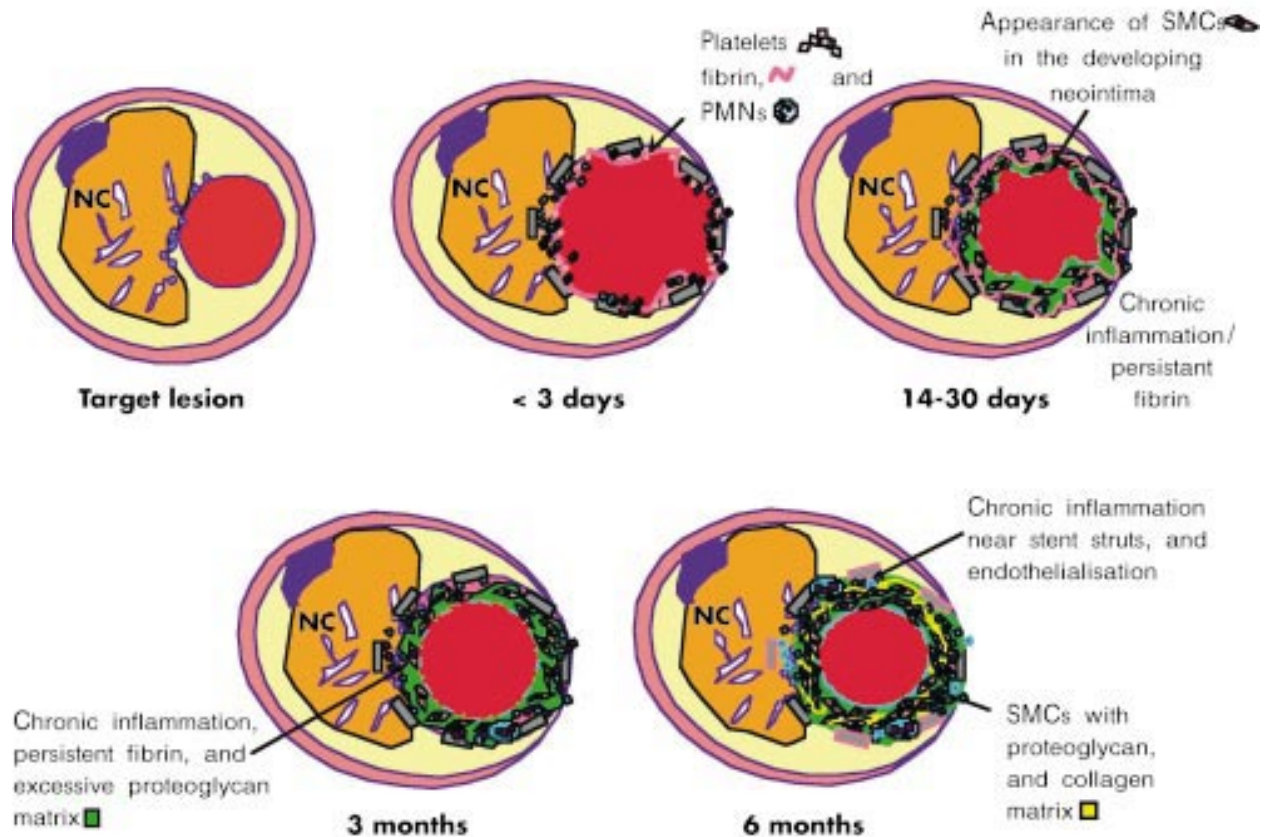


Figure 3 Illustration of the vascular response to a balloon expandable stainless steel stent implanted in an atherosclerotic human coronary artery. NC, necrotic core; PMNs, polymorphonuclear leucocytes; SMCs, smooth muscle cells.

arteries are similar to those of “bare” stainless steel stent arteries seven and 14 days, thus representing a two to three week delay in healing. Late studies at 90 or 180 days with either paclitaxel coated (pig and rabbit) or sirolimus (pig) coated stents have been negative. At this time, neointimal healing is relatively complete; fibrin and inflammation are absent, and the luminal surface is fully endothelialised. Notably, elution profiles of sirolimus eluting stents in pig coronary arteries show that 63% of the initial dose (196 (9) $\mu\text{g}/\text{stent}$) is eluted by 14 days; at this time, arterial wall concentrations are at maximum (around 160 μg) and are reduced by 50% by 28 days.²⁶ The loss of sustained efficacy may simply be an effect of an insignificant amount of drug on the stent. Alternatively, persistent fibrin deposition within the first one to two months may lead to the formation of fibrin split products which act as a stimulus for smooth muscle cell proliferation.²⁷

CLINICAL STUDIES WITH DRUG ELUTING STENTS: A COMPARISON WITH ANIMAL MODELS

In the initial clinical study of sirolimus coated BX Velocity stents in 30 patients with fast release (< 15 days) or slow release (≥ 28 days) implants, there was minimal neointimal hyperplasia at four months detected by ultrasound and quantitative angiography.³ No major adverse cardiac events (MACE)—defined as stent thrombosis, repeat revascularisation, myocardial infarction, or death—occurred up to eight months. In the larger multicentre, randomised, double blind RAVEL trial (randomised study with the sirolimus eluting velocity balloon expandable stent in the treatment of patients with de novo native coronary artery lesions) comparing sirolimus eluting with standard uncoated stents in 232 patients, restenosis rates at 210 days in the sirolimus and control stent groups were 0% and 26%, respectively.⁵ The event-free survival with sirolimus

eluting stents was 94.1% and 70.9% in control group; however, only 50% of patients with document restenosis in the control group were symptomatic.

A subsequent US multicentre trial, SIRIUS (sirolimus eluting stent), was established to test the efficacy of sirolimus eluting Bx Velocity stents in more complex lesion morphologies (Leon MB, 2002; unpublished data). A preliminary analysis of 400 patients at eight months showed an in-stent restenosis rate of 2.0% and 31.1% with sirolimus eluting and uncoated control stents, respectively. The peri-stent restenosis rates (5 mm proximal and distal outside margins of the stent), however, were similar in the two groups; thus in-segment restenosis (within both margins and stent) reached a maximum of 9% and 32% in sirolimus and uncoated stents. At nine months, MACE rates were significantly less in sirolimus eluting stents (8.9%) than in controls (18.9%), with notable reductions in target vessel revascularisation of 4.7% and 16.7%, respectively. Thus a 0% restenosis rate with sirolimus eluting Bx Velocity stents is unlikely, as these devices are used in more complex and challenging coronary lesions. Further, the failure to suppress neointimal hyperplasia at the stent margins with sirolimus may become a critical issue, particularly in smaller vessels.

Intravascular ultrasound (IVUS) findings in a subset of 95 patients from the RAVEL trial showed an equally remarkable reduction in neointimal hyperplasia (2 (5) mm^3 v 37 (28) mm^3) and per cent stent volume obstructed (1 (3)% v 29 (20)%) in sirolimus and control stents, respectively, at six months.⁶ It is important to emphasise, however, that the negligible increase in neointimal growth with sirolimus stents suggest that many of the struts remain uncovered as a result

of incomplete healing, which raises doubts as to whether restenosis was truly prevented rather than just delayed. In addition, patients receiving sirolimus eluting stents showed a significant 21% incidence of malapposition of the stent to the arterial wall, compared with only 4% in controls. This finding may represent positive remodelling of the vessel with accumulated thrombus between the stent struts and vessel wall, which would not be visualised by IVUS. Careful long term follow up by IVUS is necessary to clarify the significance and aetiology of malapposition with sirolimus eluting stents.

Extremely low rates of restenosis (0–5%) at six and 12 months have similarly been observed for paclitaxel eluting stents, compared with 11–27% in control patients (unpublished results, 2002: TAXUS I (feasibility study evaluating safety of the NIRx paclitaxel coated conformer coronary stent for the treatment of de novo coronary lesions) and ASPECT (Asian paclitaxel eluting stent clinical trial)). MACE rates were 0% in the paclitaxel eluting stent group versus $\geq 7\%$ in controls. As with sirolimus, the extent of healing of the intimal surface overlying paclitaxel eluting stents in humans is unknown. A recent study, however, of 15 patients treated for in-stent restenosis with QuaDS stents (Quanam Medical Corporation) containing the paclitaxel derivative 7-hexanoyltaxol (QP2 or “taxen”) may provide some insight into stent healing in humans.²⁸ Although at six months there was minimal in-stent intimal hyperplasia (late loss, 0.47 (1.01) mm), at 12 months there was an aggressive increase in neointimal growth (late loss, 1.36 (0.94) mm), resulting in a dramatic 61.5% rate of restenosis. Morphological examination of atherectomy tissue from a subset of these patients showed persistent fibrin admixed with smooth muscle cells and extensive proteoglycan matrix, thus demonstrating incomplete neointimal healing even at 12 months (Virmani R, 2002; unpublished data).

At the very least, the data from the QuaDS-QP2 stent emphasise the fact that late neointimal regrowth cannot be ruled out a priori with any drug eluting stent. Further, these histological results of restenotic tissue from QuaDS-QP2 stents are strikingly similar to those in animal models with paclitaxel eluting stents, even though the time course of healing is more prolonged in humans. Finally, the results of the QuaDS-QP2 stents and the terminated trial of actinomycin D eluting stents indicate that antiproliferative and anti-inflammatory drugs will not all uniformly show a drug class effect for the successful prevention of in-stent restenosis.

CONCLUSIONS

Animal models of stenting probably predict human responses, as the stages of healing are remarkably similar. What is characteristically different is the temporal response to healing, which is substantially prolonged in humans. We therefore postulate that the prevention of restenosis in recent clinical trials of drug eluting stents represents a near absent or incomplete phase of intimal healing. To this point, the negative findings of drug eluting stents in 90 and 180 day animal studies—at a time when healing is complete—may correspond to a reasonable approximation of two to three years in humans. Continued long term follow up of patients with drug eluting stents for MACE and angiographic restenosis is therefore imperative. At best, drug eluting stents may have solved the in-stent restenosis problem; at worse, they may lead to adverse long term late thrombosis and restenosis. An intermediate result would be delayed restenosis, which would improve the interim quality of life and provide time for the development of novel treatments aimed at sustained neointimal suppression. It is extremely important to emphasise, however, that the recent clinical success of drug eluting stents should not create unfounded prejudices against animal models. Although they do not exactly simulate human in-stent restenosis, they are essential for the assessment of

efficacy and safety of interventional devices and provide useful information on the pathology of arterial healing responses to antirestenotic drugs.

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IMAGES IN CARDIOLOGY.....

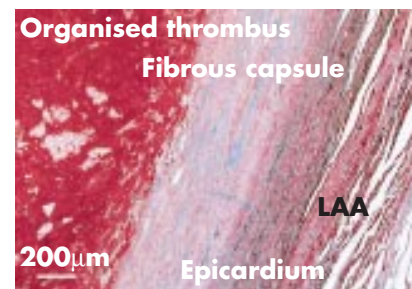
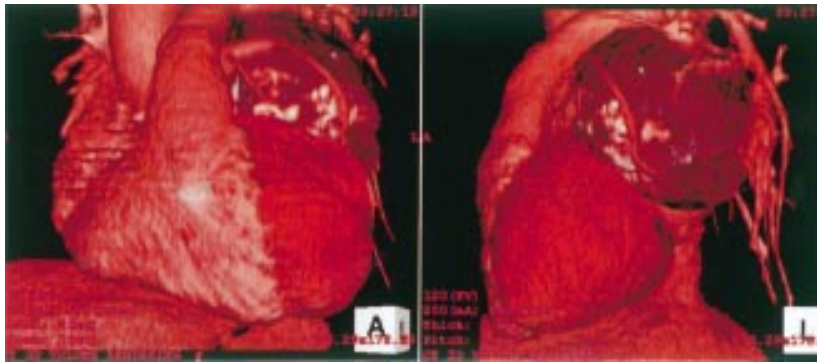
Huge mass bordering on left atrium raises coronary artery

A 45 year old carpenter with an abnormal chest radiograph was referred to us. Computed tomographic (CT) chest scan showed a round mass, 7.5 cm in diameter, bordering on the left atrium with patchy calcification around it. Density inside was almost homogeneous and no effect of the enhancement was observed. Three dimensional reconstruction from the CT showed that the left circumflex artery was raised by the mass and its proximal side dilated (lower panels, left and centre). Coronary angiography did not detect any feeding artery to the mass, and laboratory tests including tumour makers showed no abnormality. The mass was surgically enucleated.

Histological study revealed the mass to be an encapsulated pseudocyst beneath the pericardium and contained the old thrombus (lower panel, right). The precise mechanism of this thrombus formation was unknown, but might be related to blunt trauma received during the patient's work.

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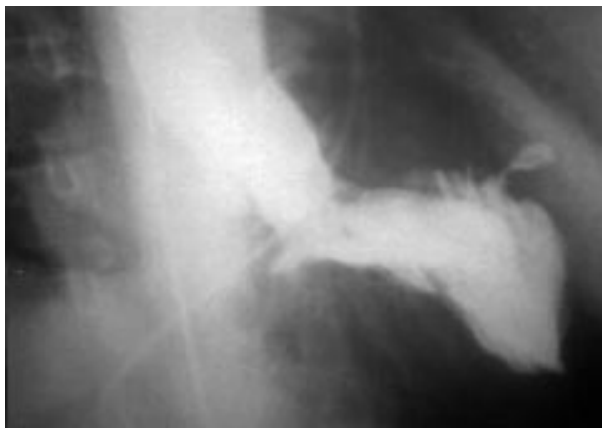
Acute left ventricular rupture after myocardial infarction

A 71 year old woman with a two hour history of chest pain and ST segment elevation in leads V1–V4 was admitted to our hospital with the diagnosis of acute myocardial infarction and treated with tissue plasminogen activator. Forty eight hours after admission, the patient presented sudden transient hypotension without other symptoms. Transthoracic echocardiography, performed immediately after the onset of hypotension, showed a small amount of pericardial effusion. Left ventricular angiography showed a digital false aneurysm

(below left) in the left anterior ventricular wall. In the operating room 200 ml of haemopericardial effusion were evacuated and left ventricular rupture was confirmed. The patient underwent simple patch covering of the wall rupture (below right) and left anterior descending artery bypass grafting. The patient returned to normal activity.

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Arterial dysfunction in syndrome X: results of arterial reactivity and pulse wave propagation tests

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Objective: To assess arterial distensibility using pulse wave velocity (PWV) measurements and its relation with endothelium dependent vasodilatation (EDV) in patients with cardiological syndrome X.

Methods: The study group consisted of 92 patients: 52 with syndrome X (34 women, 18 men, mean (SD) age 45 (3) years) and 40 healthy volunteer controls (27 women, 13 men, mean (SD) age 41 (2) years) without risk factors of atherosclerosis and with negative ECG exercise test and normal proximal coronaries on transoesophageal echocardiography. Patients with arterial hypertension, diabetes mellitus, valvar disease, or cardiomyopathy were excluded. PWV measured by a Complior Colson device was calculated for each patient. EDV was assessed from two dimensional Doppler measurement using an Acuson Sequoia with 8 MHz linear transducer at rest, during postischaemic reactive hyperaemia, and after an oral dose of 400 µg of glyceryl trinitrate.

Results: PWV was significantly higher in patients with syndrome X than in healthy subjects (9.3 (0.7) m/s v 8.2 (0.9) m/s, respectively, $p < 0.001$). Baseline brachial artery diameter was similar in the syndrome X and control groups (4.0 (0.6) mm v 4.08 (0.64) mm, NS). EDV was impaired in patients with syndrome X compared with controls (6.6 (3.0)% v 11.1 (3.9)%, $p < 0.001$). Endothelium independent vasodilatation was similar in both groups. In patients with syndrome X there was a positive correlation between PWV and the degree of EDV ($r = 0.864$, $p < 0.001$). The cut off value for PWV was 8.5 m/s, with a sensitivity of 62% and a specificity of 91%.

Conclusions: EDV but not glyceryl trinitrate induced vasodilatation is decreased in patients with syndrome X. There is a strong correlation between PWV and the degree of endothelial dysfunction of peripheral arteries in patients with syndrome X. PWV assessment may be useful to identify abnormal vascular physiology in these patients.

Approximately 10% of coronary arteriograms performed for the assessment of patients with chest pain show normal coronary anatomy.¹⁻³ Chest pain is the hallmark of syndrome X, but its aetiology in this group of patients appears to be heterogeneous and remains unclear. Patients with syndrome X are characterised by the presence of chest pain, ST segment depression, and a positive ECG exercise test but normal coronary angiogram.⁴ The underlying mechanism of ischaemia in these patients has not been described. An abnormal coronary flow reserve may indicate that the observed symptoms in syndrome X have a microvascular origin.⁵ Invasive methods of assessing endothelium dependent and independent vasomotor responses of coronary arteries have shown that vasodilation in response to pharmacological or metabolic agents is impaired.⁶ Abnormal findings of myocardial perfusion in syndrome X can be explained by an impaired vasodilation capacity of coronary arteries.⁷ A common finding in the majority of patients with syndrome X is endothelial dysfunction of the coronary arteries.⁸ After supplementation with L-arginine, improvement in endothelium dependent vasodilation (EDV) was observed, which suggests that impaired nitric oxide synthesis may have a key role in the development of symptoms in syndrome X. However, some studies failed to show such a relation, indicating that there are defects in adenosine metabolism.⁹ The correlation between EDV of coronary and brachial artery vasomotor responses, especially in patients with coronary artery disease, has been described.^{10,11} Findings of Teragawa and colleagues¹² suggest that flow mediated dilatation of the brachial artery may be used to detect the presence of coronary artery disease in patients suspected of having it. Abnormal brachial dilatation has also been observed in patients with microvascular angina or with coronary spastic angina.^{13,14}

The speed of pulse wave propagation throughout the arterial tree depends on the elastic and geometric properties of the arterial wall. Since blood is an incompressible fluid enclosed in the system of elastic arteries, the energy released by ventricular ejection is propagated mainly along the arterial wall. An increase in pulse wave velocity (PWV) may be used as an early indicator of arterial stiffening, characteristic of the development of atherosclerosis.¹⁵ The aim of this study was, by assessing endothelial function of the brachial artery, to investigate whether endothelial dysfunction in patients with syndrome X is a generalised process. To examine the systemic vascular bed we also measured pulse wave propagation as a marker of arterial distensibility. Our secondary objective was to investigate a possible correlation between endothelial function and PWV measurements in patients with syndrome X.

METHODS

Patient population

The study population consisted of 52 patients with angina, without prior myocardial infarction (mean (SD) age 45 (3) years, 34 women, 18 men) who underwent coronary angiography in our cardiology department during 1998 and 1999. They fulfilled the characteristic criteria for the diagnosis of syndrome X: angina-like chest pains, positive ECG exercise test, normal coronary arteries on angiography, and no metabolic disorders.

Exclusion criteria were a previous diagnosis of dilated or constrictive cardiomyopathy, a previous myocardial infarction,

Abbreviations: EDV, endothelium dependent vasodilatation; GTN, glyceryl trinitrate; PWV, pulse wave velocity

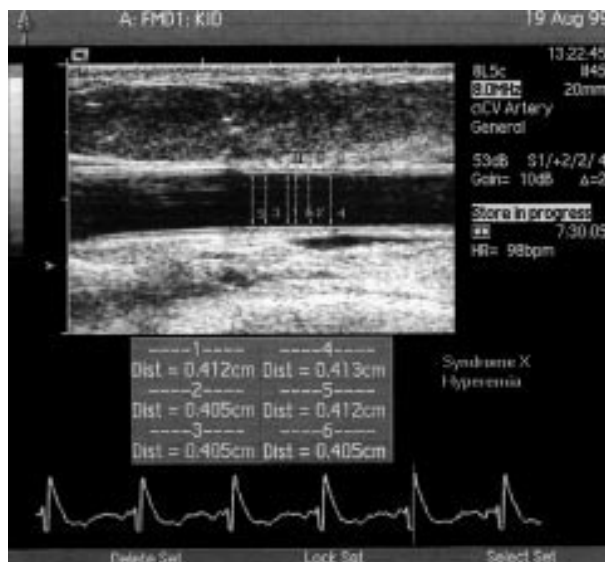


Figure 1 Brachial artery scans after reactive hyperaemia. During each examination the result was calculated as the mean of six measurements.

arterial hypertension, metabolic disorders such as diabetes mellitus, dyslipidaemia, and insulin resistance syndrome, ECG conductivity and rhythm disorders, valvar heart disease, Barlow's syndrome, or any other known functional peripheral arterial disorders. None of our patients was treated at the time of examination with angiotensin converting enzyme inhibitors, long acting nitrates, statins or hormone replacement therapy.

Forty healthy volunteers (mean (SD) age 41 (2) years, 27 women, 13 men) without any symptoms or risk factors of coronary artery disease and with a normal ECG and echocardiogram were enrolled in the control group. Each patient from the control group underwent an ECG exercise test, which was negative. Coronary angiography was not performed in this group for ethical reasons. Before enrolment in the control group, each patient underwent transoesophageal echocardiographic examination of proximal parts of the coronary arteries. Those with abnormal findings in transoesophageal echocardiography or turbulent flow suggesting the presence of atherosclerosis in the coronary arteries were excluded from the study.

Study protocol

An exercise test (Bruce protocol) was said to be positive if there was at least 0.2 mV of horizontal ST segment depression or elevation in at least two leads. The ECG exercise test was aborted if the heart rate reached submaximal values, patients were complaining of chest pain, complex forms of cardiac arrhythmias were observed, or criteria for a positive ECG test were reached.

Blood samples were taken from each patient and analysed for serum urea, electrolyte, cholesterol, and triglyceride concentrations.

Echocardiographic assessment was performed in all patients according to the standards of the American Society of Echocardiography, with measurements of diastolic and systolic septal and posterior wall thickness, left ventricle diameters, and ejection fraction.

Coronary angiography was performed in patients with syndrome X using the standard Judkins technique on a General Electric Advantax LX system (General Electric Medical Systems, Milwaukee, Wisconsin, USA). All images were digitally stored in DICOM format on an Hewlett Packard Visualise workstation (Hewlett-Packard Co, Palo Alto, California, USA) for further analysis.

Endothelial function was assessed with an 8 MHz linear array transducer and Acuson Sequoia 256 echocardiographic

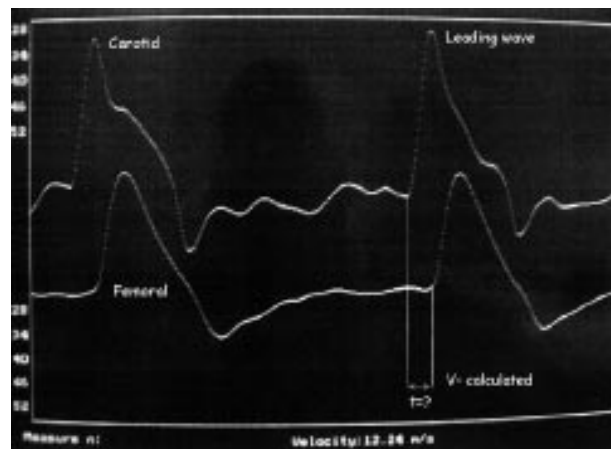


Figure 2 Pulse wave measurement in patients with syndrome X.

system (Acuson, Mountain View, California, USA) according to the protocol described by Fathi and colleagues¹⁶ and Celermajor and associates.¹⁷ The right brachial artery was scanned at rest, during reactive hyperaemia, and after administration of sublingual glyceryl trinitrate (GTN). Before the first scan the patient rested in the supine position for at least 10 minutes. Then the brachial artery diameter and blood flow velocity were measured. Blood flow was measured from the pulsed Doppler signal at a maximum incidence angle of 60°, with the Doppler gate positioned in the middle of the arterial lumen. Reactive hyperaemia was induced with inflation of a pneumatic tourniquet to a pressure of 200 mm Hg for five minutes. The artery diameter was measured 45–60 seconds after cuff deflation and flow velocity recordings were started 15 seconds after cuff release. After 15 minutes an additional baseline scan was recorded and then four minutes after 400 µg sublingual GTN was given, the last images were collected. ECG monitoring was continuous during the procedure, and ECG R wave measurements were averaged from four consecutive beats. Arterial diameters were measured from the anterior to the posterior "m" line at the end of diastole. All measurements were taken by two observers who were unaware of clinical details. The previously estimated mean (SD) inter-sonographer variability of flow mediated dilatation measurement in our echocardiography laboratory was 1.2 (0.4)%.

Vasodilatation was expressed as the percentage increase in artery diameter during reactive hyperaemia (EDV) and after GTN administration (endothelium independent vasodilatation). Reactive hyperaemia was calculated as the maximum flow over the 15 seconds after cuff release divided by the baseline flow values (fig 1).

PWV was measured automatically with a Complior Colson device based on an IBM Aptiva 486 DX personal computer according to the methods described by Asmar and colleagues.¹⁸ PWV was calculated from measurements of the pulse transit time between two recording sites, the femoral and carotid external arteries, at a sampling frequency of 500 Hz. During preprocessing analysis the gain of each waveform was adjusted to obtain an equal signal for the two waveforms. During PWV measurements, after pulse waveforms of sufficient quality were recorded, the digitisation process was initiated by the operator and automatic calculation of the time delay between two upstrokes was started (fig 2). The interobserver repeatability (repeatability coefficient) for the automatic PWV calculation in our laboratory was 0.876 (95% confidence interval ± 1.95 repeatability coefficient).

Statistical analysis was performed with the Statistica 5.0 data analysis program using non-parametrical U Mann-Whitney tests. Correlations were calculated with the Spearman test. All results are expressed as mean (SD).

The investigation conforms with the principles outlined in the Declaration of Helsinki.

Table 1 Basic data in patients with syndrome X and the control group

	Syndrome X group	Control group	p Value
Number of patients	52	40	NS
Women	34	27	NS
Average age (years)	45 (3)	41 (2)	0.01
Smokers	3	0	0.01
Positive family history for IHD	5	0	0.01
Systolic blood pressure (mm Hg)	124.7 (7.9)	124.8 (7.2)	NS
Diastolic blood pressure (mm Hg)	77.1 (6.3)	77.1 (6.2)	NS
Total cholesterol (mmol/l)	4.72 (0.19)	4.68 (0.22)	NS
LDL cholesterol (mmol/l)	2.72 (0.32)	2.71 (0.33)	NS
HDL cholesterol (mmol/l)	1.52 (0.14)	1.53 (0.15)	NS
Triglycerides (g/l)	1.04 (0.12)	1.01 (0.13)	NS
Resting heart rate (beats/min)	75.6 (7.8)	71.0 (9.7)	0.01
Maximum heart rate (beats/min)	151 (10)	164 (12)	0.01
Maximum ST depression (mm)	2.4 (0.4)	0.9 (0.4)	0.01
Exercise test duration (min)	6.8 (1.8)	9.6 (0.9)	0.01
Ischaemic pain present (number)	29	0	NA

Values are mean (SD). HDL, high density lipoprotein; IHD, ischaemic heart disease; LDL, low density lipoprotein; NA, not applicable; NS, not significant.

RESULTS

Table 1 shows demographic and biochemical data.

Because of the enrolment criteria, patients in the control group were younger than those in the syndrome X group (mean (SD) 41 (2) years *v* 45 (3) years, respectively) to minimise the risk for silent coronary disease.

Blood analysis showed significantly higher plasma concentrations of total cholesterol, low density lipoprotein fraction, and triglycerides in the syndrome X population than in controls. High density lipoprotein concentrations were much lower in the syndrome X group. However, all of these values were within the normal ranges (table 1).

ECG exercise tests were positive only in patients with syndrome X. In the syndrome X group, baseline heart rate was significantly higher and total exercise phase was significantly shorter than in the controls (table 1). In 29 patients with syndrome X, chest pain occurred during the test at a mean (SD) exercise time of 4.9 (1) minutes.

Echocardiographic examination confirmed that both groups had normal cardiac size and function with clinically insignificant differences between the groups. The ejection fraction was slightly lower in patients with syndrome X than in the control group.

Arterial vasodilator tests

Under baseline conditions there were no significant differences in measurements of brachial artery diameter (4.0

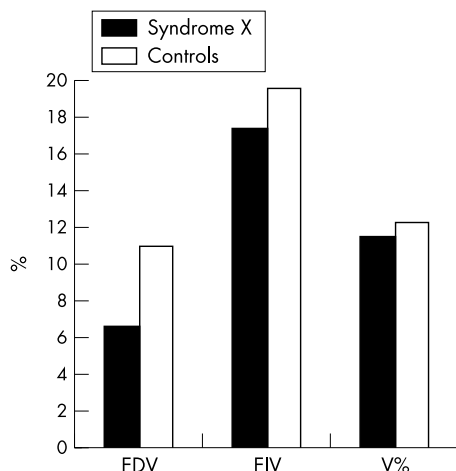


Figure 3 Comparison of mean increase in brachial artery diameter after reactive hyperaemia between the syndrome X and control groups.

(0.6) mm *v* 4.08 (0.64) mm, NS) and baseline blood flow velocities (74.1 (14.2) cm/s *v* 73 (13.9) cm/s, NS) between the syndrome X and control groups, respectively. After oral administration of GTN the mean increase in blood flow velocity and in arterial diameter was similar in both groups and there was no significant difference in endothelium independent vasodilatation (mean (SD) percentage arterial diameter increase 18.1 (5.1)% *v* 19.6 (5.1)%, NS).

After the induction of reactive hyperaemia the mean (SD) increase in arterial diameter was significantly lower in patients with syndrome X than in controls (0.27 (0.12) mm *v* 0.44 (0.16) mm, respectively), which showed that EDV was significantly impaired in patients with syndrome X (6.6 (3.0)% *v* 11.1 (3.9)% in controls, $p < 0.001$) (fig 3).

During hyperaemia the increase in blood flow velocity was similar in the syndrome X and control groups.

PWV measurements

In the syndrome X group pulse wave propagation time between measurement sites was significantly shorter than in the control group (65.1 (6.2) ms *v* 76.1 (8.2) ms, respectively, $p < 0.001$).

Comparing with the syndrome X group, PWV values were significantly lower in controls (9.3 (0.7) m/s *v* 8.2 (0.9) m/s, $p < 0.001$), as fig 4 shows.

A PWV of 8.5 m/s allows for differentiation between groups with a sensitivity of 62% and a specificity of 91%.

Further analysis showed the presence of a strong inverse correlation between PWV and EDV (fig 5). The regression equation is $PWV = 15.07 - 0.75 \times EDV$, Pearson's $r = -0.864$, $p < 0.01$.

In our study we did not find any significant correlation between PWV and age, sex, or mean blood pressure in the studied groups.

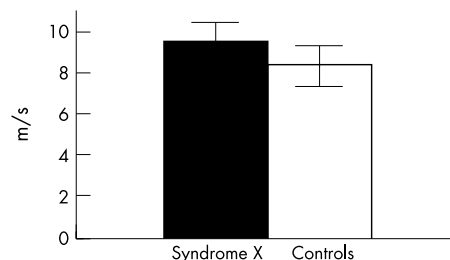


Figure 4 Mean (SD) pulse wave velocity (m/s) measurements in the study groups.

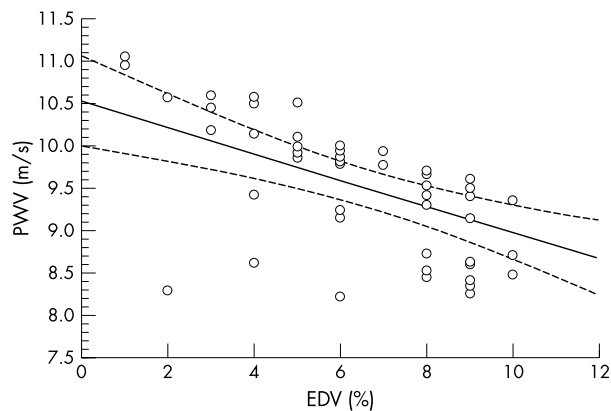


Figure 5 Correlation between pulse wave velocity (PWV) and endothelium dependent vasodilatation (EDV), where $PWV = 15.07 - 0.75 \times EDV$, Pearson's $r = -0.864$, $p < 0.01$.

DISCUSSION

Our study showed two main abnormalities in peripheral arterial function in patients with syndrome X: impaired EDV and increased PWV. The response to nitrates was not altered, indicating that endothelium independent vasodilatation is not impaired in patients with syndrome X.

In previous studies reduced acetylcholine induced vasodilatation of coronary arteries in syndrome X was reported.¹⁹ Endothelial dysfunction in coronary arteries may lead to reduction in arterial flow reserve and induce ischaemic pain in syndrome X. This theory may be supported by the results of endothelium vasodilator tests performed with intracoronary acetylcholine infusions, which showed an abnormal constrictive reaction especially in sites with increased risk for development of atherosclerosis such as proximal parts or bifurcation of the arteries in patients with syndrome X.^{6-8, 19}

Several conditions such as hypercholesterolaemia, hyperglycaemia, low high density lipoprotein cholesterol, acute postprandial hypertriglyceridaemia, active and passive cigarette smoking, susceptibility of low density lipoprotein to oxidation, hypertension, and early stages of atherosclerosis are associated with impaired EDV.^{20, 21} In studies performed by Celermajer and colleagues,^{22, 23} they observed a strong correlation between atherosclerotic risk factors and the degree of endothelial dysfunction. Anderson and colleagues²⁴ showed that brachial artery vasodilator tests correlated with measures of coronary endothelial function.

Results of our study indicate that impaired endothelial function in syndrome X is likely to be a generalised process involving the peripheral conduit arteries and may be similar to that observed in atherosclerotic heart disease.¹¹ Evidence of generalised endothelial dysfunction in syndrome X was also described in other studies.²⁵ In some patients with impaired flow mediated vasodilatation defects of thallium 201 distribution were also described; moreover, endothelial function in these patients was restored after administration of L-arginine, indicative of nitric oxide synthesis defects in syndrome X.⁷

Pathogenesis of chest pain in syndrome X is not well explained. The absence of atherosclerotic changes in coronary angiography cannot exclude the possibility of intramural plaques. As described in an intravascular ultrasonography study by Erbel and colleagues,²⁶ despite the lack of stenoses in coronary angiography, abnormal changes in coronary artery walls were common, usually increased intima thickness, although small intramural atherosclerotic plaques were also found.

Our second finding in syndrome X was an increased PWV, reflecting increased arterial stiffness. A close relation between increased PWV and atherosclerosis development has been reported.^{27, 28} Measurements in patients with coronary artery disease showed decreased compliance of large arteries. To the

best of our knowledge, PWV has not been assessed before in patients with syndrome X. In our study we found a significant difference in PWV between patients with syndrome X and healthy controls, and we established a cut off value of PWV that is useful in differentiating these patients with satisfactory specificity and sensitivity. PWV measurements provide highly reproducible estimates of arterial distensibility, particularly with the automated method proposed by Asmar and colleagues.¹⁸ Some studies suggest that the PWV increase may be used as an early indicator of atherosclerosis development, and a significant decrease in compliance in large arteries was found in patients with atherosclerosis risk factors such as heterozygous familial hypercholesterolaemia.²⁹

A significant correlation between increased arterial stiffness and atherosclerosis formation was described. In some studies, however, hypertension was shown to influence arterial rigidity more than arteriosclerotic risk factors.³⁰ All patients selected for our study were normotensive so as to minimise the effect of arterial remodelling caused by increased arterial pressure.

EDV impairment in our patients with syndrome X was closely correlated with PWV values. Our results indicating impairment of endothelial function and decreased arterial distensibility may support the theory that the vascular abnormalities in syndrome X are a generalised process similar to those observed in the early stages of atherosclerosis.

Study limitations

We did not undertake an intravascular ultrasound examination, the ultimate means of visualising coronary vascular anatomy. However, our intention was to test non-invasive techniques for analysing the function of systemic arteries in syndrome X. Even with intravascular ultrasound technology, subtle anatomical abnormalities in distal parts of the coronary arteries may be undetectable. The age difference between groups may also have influenced our results, but we did not find a correlation between PWV and age in the studied patient populations, nor any correlation between endothelial function and age.

The results of our study do not provide firm evidence that there is a direct link between increased PWV and myocardial perfusion impairment in syndrome X, although the presence of a correlation between PWV and the degree of endothelial dysfunction may indicate such a relation. The cut off value of PWV was measured retrospectively and further prospective studies should be performed to investigate the usefulness of this parameter in identifying patients with syndrome X.

Conclusions

EDV is impaired in peripheral arteries of patients with syndrome X, and a significant rise in PWV reflects the increased arterial wall stiffness in such patients. A strong inverse correlation between PWV and EDV may indicate generalised arterial dysfunction in these patients. PWV measurements may be useful in differentiating patients with syndrome X from healthy subjects.

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Erratum

Sanchez PL. Acute left ventricular rupture after myocardial infarction (Images in cardiology). *Heart* 2003;**89**:138.

The following co-authors should have been included in the author list: Arribas A, Gonzalez-Santos JM, Collado JR. The error is regretted.