

SCIENTIFIC LETTERS

Transforming growth factor- β_1 expression in dilated cardiomyopathy

Transforming growth factor- β_1 (TGF- β_1) is a multifunctional cytokine that has an important role in the regulation of cell growth, differentiation, and repair in a variety of tissues.¹ In addition to its role in the cell cycle and apoptosis, TGF- β_1 induces the synthesis of extracellular matrix (ECM) and is upregulated by angiotensin II.² Dilated cardiomyopathy is characterised by myocyte loss, hypertrophy of residual myocytes, increased interstitial fibrosis, and abnormalities of the cytoskeleton.³ Cytotoxic lymphocytes and macrophages are also present in the myocardium in increased numbers which may provide a source of TGF- β_1 .⁴ Therefore in this study we have investigated whether patients with chronic heart failure caused by idiopathic dilated cardiomyopathy have increased plasma concentrations of TGF- β_1 , and whether this is associated with increased

macrophage gene expression for TGF- β_1 , compared to those with normal left ventricular function. Patients with ischaemic or hypertensive heart disease were excluded because of the potential confounding effects on TGF- β_1 , concentrations of atherosclerosis and pressure overload with left ventricular hypertrophy.

Twenty patients who presented with symptoms and signs of chronic heart failure with no obvious cause and with the clinical diagnosis of dilated cardiomyopathy (by the World Health Organization criteria) were studied. All patients had right and left cardiac catheterisation with standard haemodynamic measurements, and coronary angiography was performed to exclude significant valvar, coronary artery disease, hypertensive heart failure, as well as constrictive pericardial disease or restrictive cardiomyopathy before proceeding to right ventricular biopsy. Twenty age and sex matched healthy controls with no evidence of cardiac disease (all with normal echocardiograms) were also studied. Serum TGF- β_1 was assayed with an enzyme linked immunosorbent assay (ELISA) (R & D System, Minneapolis, USA) The intra- and interassay coefficient of variation was < 15%. Peripheral blood mononuclear cells were isolated and were resuspended in medium for total cell counting. CD14 cells were isolated using dynabeads M-450 CD14 (Dyna, AS,

Oslo, Norway). The cells were incubated at 37°C in a humidified 5% carbon dioxide atmosphere, in the presence of phytohaemagglutinin (10 μ g/ml) and Phorbol 12-myristate 13 acetate (1 ng/ml). The cells were harvested at time 0 and 24 hours after stimulation for total RNA extraction. After RNA extraction TGF- β_1 gene expression was quantified by the PCR MIMIC method (Clontech, Palo Alto, California, USA). Clontech, primers sequences are: 5' GCC CTG GAC ACC AAC TAT TGC T 3' and 5' AG GCT CCA AAT GTA GGG GCA GG 3'. The PCR product generated from MIMIC fragment has 270 bp while those from target cDNA has 161 bp, and were separated by 1.7-2% agarose gels and stained with ethidium bromide. Densitometry was performed and a standard curve was established by plotting the log value of the band intensity ratio of the MIMIC fragment and the target fragment with log MIMIC molecule number. The initial target molecule number can be calculated and is equal to the MIMIC molecule number when the log band intensity ratio is zero. The value was normalised as cDNA molecule per cell or gram of tissue.

The results showed that serum TGF- β_1 concentrations were higher in the dilated cardiomyopathy group (mean (SE) 28.4 (2.2) ng/ml) compared to normals (14.7 (1.6) ng/ml; $p = 0.0001$, Mann Whitney test). The individual values are shown in fig 1. There was a significant increase in macrophage TGF- β_1 mRNA in dilated cardiomyopathy patients compared to controls (18.9 (6.0) *v* 1.54 (0.5) $\times 10^{-3}$ molecules/cell; $p = 0.006$). Individual values are also shown in fig 1. There was no relation between TGF- β_1 mRNA, and ejection fraction ($r = 0.027$; $p = 0.5$), clinical course over the previous 6-24 months as assessed by the change in ejection fraction ($r = 0.005$; $p = 0.77$), or left ventricular diastolic function (TGF- β_1 mRNA expression in those with a restrictive left ventricular filling pattern on echocardiography was the same as those without: 2.02 (0.29) and 2.05 (0.26), respectively).

In response to injury or disease, the production of TGF- β increases cell proliferation and ECM production is stimulated to repair and heal the tissue'. This is achieved through simultaneously stimulating the synthesis of ECM proteins, inhibiting the actions of proteases that degrade ECM, and increasing receptors on cell surfaces. Increased concentrations of TGF- β , produced by injury, injecting TGF- β_1 or transferring the TGF- β_1 gene invariably leads to tissue fibrosis.

TGF- β_1 may be especially important in dilated cardiomyopathy in which there is an overall increase in intramyocardial fibrillar collagen. Recently, Pauschinger presented results in 18 patients with dilated cardiomyopathy suggesting that the gene expression of collagen type III correlated with the gene expression of TGF- β_1 in myocardial biopsy specimens, although they did not identify the cell type responsible.⁵ However, ours is the first published report to demonstrate in patients with idiopathic dilated cardiomyopathy increased macrophage gene expression for TGF- β_1 associated with increased circulating concentrations.

It is well documented in patients with idiopathic dilated cardiomyopathy that cytotoxic lymphocytes and macrophages may be present in significant numbers within the myocardium, which is not apparent using

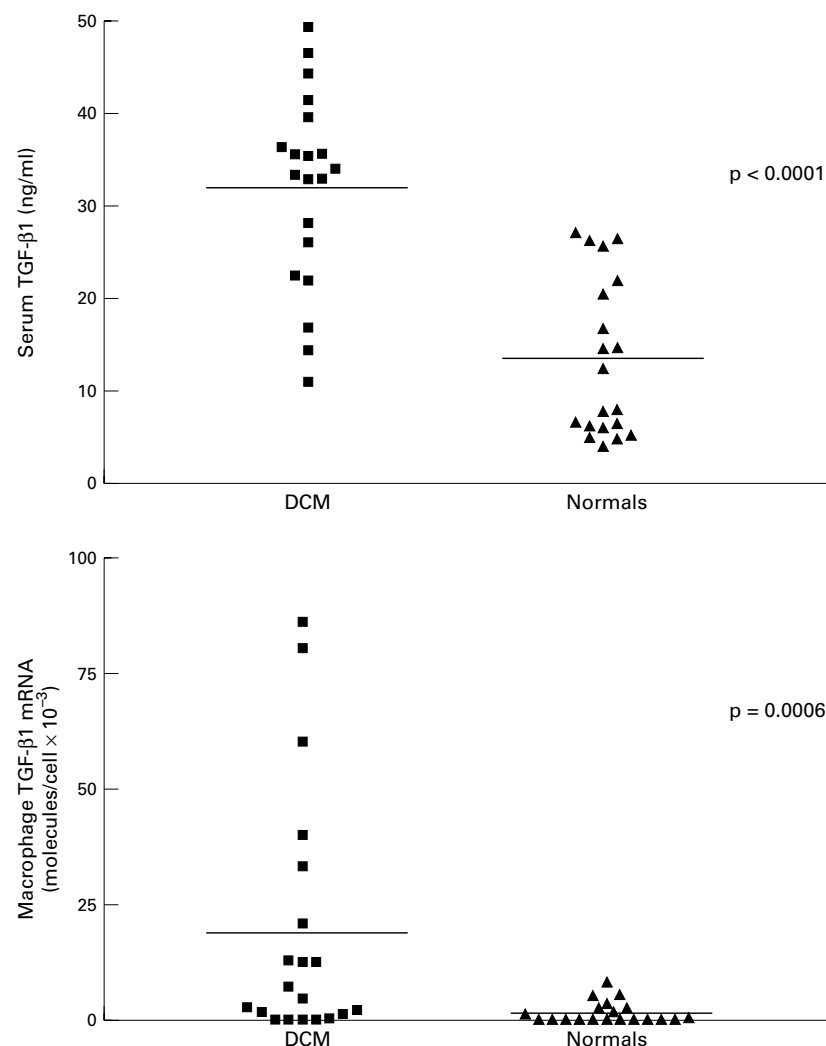


Figure 1 Scattergram showing individual values for serum TGF- β_1 concentrations (upper) and macrophage mRNA concentrations (lower). DCM, idiopathic dilated cardiomyopathy.

light microscopy and requires electron microscopy or immunohistologic techniques.⁴ Using special immunohistologic staining methods we have found that approximately 40% of myocardial biopsy specimens have increased lymphocytes and macrophages, similar to the published results.⁴ However, we have not been able to detect any enteroviral genome in right ventricular endomyocardial biopsy specimens from this group of patients (unpublished data). Although macrophages are recognised to be important mediators of cardiac injury, repair, and TGF- β , there may be other cellular sources for the raised plasma TGF- β , from within the myocardium, and in situ hybridisation PCR studies are required to define precisely which particular cells are the source.

In summary we have shown, for the first time, increased gene expression for TGF- β , in macrophages from some patients with idiopathic dilated cardiomyopathy associated with increased plasma concentrations. Probably this reflects the role of macrophages in tissue repair, remodelling and healing. But excessive production of TGF- β , may account for the increased collagen deposition found in dilated cardiomyopathy, which impairs ventricular compliance and diastolic function, and worsens heart failure.

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Idiopathic dilated cardiomyopathy: lack of association with haemochromatosis gene in the CARDIGENE study

The hereditary haemochromatosis gene (HFE) has recently been proposed as a disease modifying gene.¹ The rationale is that two common mutations of the HFE gene (C282Y and H63D) are found in a majority of patients with genetic haemochromatosis who are either homozygotes (C282Y/C282Y) or compound heterozygotes (C282Y/H63D).

Table 1 Distribution of genotypes for each polymorphism in cases and in control subjects

Genotype	+/+	C282Y/+	H63D/+	C282Y/H63D
Cases (n=418)	246 (58.9 %)	36 (8.6 %)	127 (30.4 %)	9 (2.2 %)
Controls (n=372)	228 (61.3 %)	30 (8.1 %)	106 (28.5 %)	8 (2.2 %)

Frequencies of genotypes are indicated in absolute numbers, and percentages in parenthesis.

These mutations have been shown to contribute to more subtle modifications of iron homeostasis at the heterozygous state.² In turn, iron may predispose to myocardial damage through the production of activated oxygen species. Recently, Mahon and colleagues have reported an association between the H63D mutation and idiopathic dilated cardiomyopathy (IDCM).³ In this study, 207 unrelated white patients with dilated cardiomyopathy and 200 controls were tested for HFE C282Y and H63D mutations. An increased proportion of H63D heterozygotes was found among patients (36%) as compared to the control group (27%). No association was found with C282Y mutation and as the H63D mutation had a relatively minor effect on iron status, these authors proposed that this association may be unrelated to iron metabolism. Surber and colleagues reported a study with 161 patients diagnosed with suspicion of myocarditis or IDCM where C282Y heterozygotes IDCM patients were significantly more frequent compared to controls.⁴

We have determined the frequency of both HFE mutations in the CARDIGENE study, a case-control study of IDCM enrolled in 10 different hospitals in France (for details on the population see Tesson and colleagues⁵). There were 426 patients with IDCM (339 men, 87 women). The mean (SD) age at diagnosis was 53 (10) years, and 229 patients had undergone cardiac transplantation. Patients with chronic excess alcohol consumption were not excluded. The study control group consisted of 401 subjects free of cardiovascular disease selected from the MONICA (monitoring trends and determinants in cardiovascular disease) project in France and matched for sex and age (329 men and 72 women, mean age 46 (8) years).

HFE C282Y and H63D mutations were tested by polymerase chain reaction (PCR) and allele-specific detection.

Amplification was performed in a 96 well microtitre plate as previously described⁶; 200 ng of genomic DNA were amplified in a total volume of 25 μ l containing 20 pmol of 5' and 3' primers, 0.2 mM dNTPs, 6 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 5 pmol of each allele-specific molecular Beacon, and 0.75 U of AmpliTaq Gold DNA polymerase (ABI, France). The enzyme was heat activated at 96°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 20 seconds, annealing at 55°C for 20 seconds and extension at 72°C for 20 seconds in UNO-Thermoblock (Biometra, Göttingen, Germany). After a final denaturation at 95°C for two minutes, hybridisation with the probes was carried out at 55°C for five minutes. The emission of fluorescence was recorded in a plate fluorometer fluostar (BMG, Germany) in two wavelength systems: 480-520 nm for fluorescein (FAM) and 520-590 nm for tetramethylrhodamine (TAMRA).

Primers and allele-specific probes were synthesised by Eurogentech (Seraing, Belgium):

C282Y:

forward primer: CTGTACCCCTGGG-GAAGAGCAGAG

reverse primer: CCCAGATCACAATGAG-GGGCTGATC

mutated probe: FAM-gcgac CCACCTGG-TACGTATAT gtcgc -DABCYL

normal probe: TAMRA-gcgac CCACCTGGCAGGTATAT gtcgc -DABCYL

H63D:

forward primer: GCTTTGGGCTACGT-GGATGACCAGC

reverse primer: CCATGGAGTTCGGGG-CTCCACAC

mutated probe: FAM-gcgac TTCT ATGATGATGAGAGTC gtcgc -DABCYL

normal probe: TAMRA-gcgac TTCTATGATCATGAGAGTC gtcgc -DABCYL

DABCYL: 4-4' [dimethylaminophenylazo] benzoid acid.

Data were analysed using the SAS statistical software (SAS Institute Inc, Cary, North Carolina, USA). Hardy-Weinberg equilibrium was tested by a χ^2 test with 1 degree of freedom (df). Allele frequency was calculated by gene counting. The association of each polymorphism with the disease was tested by a χ^2 test comparing cases and controls with 2 df for H63D and 1 df for C282Y since, owing to low numbers, we pooled homozygotes and heterozygotes for the rare allele.

The association with variables characterising the severity of disease was tested similarly by comparing cases below and above the median of ejection fraction (median = 24) and left ventricular dilation (median = 40), and cases with and without cardiac transplantation.

Genotypes of both H63D and C282Y mutations could be determined in 418 cases and 372 controls. There was no significant deviation from Hardy-Weinberg equilibrium in controls as well as in cases. We did not find any association between the disease and any of the two HFE mutations (table 1). The odds ratio (95% confidence interval (CI)) for Y282 and D63 carrying were 0.94 (95% CI 0.58 to 1.52) and 0.90 (95% CI 0.66 to 1.23), respectively. Allele frequencies were similar in controls and patients (Y282: 0.051 and 0.055, D63: 0.17 and 0.18, respectively). Within the patient group, there was no relation between genotype and the severity of the disease, assessed by ejection fraction, left ventricular dilation, or cardiac transplantation.

Our results on a large and well characterised population do not confirm the implication of these two common mutations in the HFE gene as genetically predisposing factors in IDCM. These contradictory results may result from different factors including sample size, different genetic background, selection criteria including degree of cardiac dilatation or ejection fraction reduction, or bias in the recruitment of the control population. These conflicting results point out the need for large populations in the search for genes susceptible to IDCM. Future studies should therefore be undertaken with clearly defined subgroups

in order to determine the modulating influence of HFE mutations in dilated cardiomyopathy.

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Effects of cardiac resynchronisation on maximal and submaximal exercise performance in advanced heart failure patients with conduction abnormality

Atrioventricular pacing (cardiac resynchronisation) is a promising treatment for patients with advanced heart failure and conduction abnormality.¹ These patients have severely impaired exercise tolerance and this has been shown to be multifactorial. The present study examined, in detail, the effect of cardiac resynchronisation on maximal and submaximal exercise capacity as evaluated by metabolic exercise testing.

Table 1 Comparison of exercise parameters before and after cardiac resynchronisation (n=15)

	Baseline	With CR	% Change	p Value
Exercise duration (sec)	382.27 (170.90)	467.87 (177.25)	+22.4	0.04
Peak $\dot{V}O_2$ (ml/min/kg)	14.31 (3.41)	16.02 (4.01)	+11.9	0.03
$\dot{V}O_{2@AT}$ (ml/min/kg)	10.0 (1.8)	10.8 (1.6)	+8.0	0.13
OUES	517.59 (148.15)	611.97 (210.25)	+18.2	0.02
$\dot{V}E/\dot{V}CO_2$ slope	40.53 (4.26)	37.13 (5.08)	-8.4	0.02

CR, cardiac resynchronisation; OUES, oxygen uptake efficiency slope; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}E$, minute ventilation.

A consecutive series of patients from a single centre with cardiac resynchronisation therapy, able to perform baseline and follow up treadmill tests, were enrolled in the study. Inclusion criteria were New York Heart Association (NYHA) functional class III/IV heart failure on maximal medical treatment, left ventricular ejection fraction < 35%, left ventricular end diastolic dimension > 60 mm, and QRS duration > 130 ms. Patients with chronic atrial fibrillation were excluded. The protocol was approved by the hospital research and ethics committee and all subjects gave written informed consent.

Left ventricular (LV) stimulation was accomplished by a lead inserted into an LV branch of the coronary sinus. Specially designed leads were used (Medtronic 2187 or 2188, Medtronic Inc, Minneapolis, Minnesota, USA). Right atrial and ventricular leads were positioned in the right atrial appendage and right ventricular apex, respectively. All patients received cardiac resynchronisation devices (InSync, Medtronic Inc) with one atrial and two ventricular ports. Optimal atrioventricular delays were individually determined by analysing transmitral flow using Doppler echocardiography.

Exercise testing was performed before and 1-3 months after implantation. Patients underwent symptom limited maximal breath-by-breath cardiopulmonary exercise testing at baseline and follow up using the Modified Naughton protocol with continuous electrocardiographic recording. Oxygen consumption ($\dot{V}O_2$, ml/min), carbon dioxide production ($\dot{V}CO_2$, ml/min) and minute ventilation ($\dot{V}E$, l/min) were continuously measured using an automated breath by breath system (Med Graphics CPX-D, Medical Graphics Co, St Paul, Minnesota, USA). Anaerobic threshold was determined by the V slope method and confirmed by the ventilatory equivalence method. The patients were all encouraged to exercise until the respiratory exchange ratio (that is, $\dot{V}CO_2/\dot{V}O_2$) was greater than 1.0 which is a good indication that near maximal effort has been reached. Maximal exercise capacity was assessed by exercise duration and peak oxygen consumption (peak $\dot{V}O_2$). Submaximal exercise capacity was assessed in three ways: by measuring oxygen consumption at anaerobic threshold ($\dot{V}O_{2@AT}$); by examining continuous oxygen uptake using the oxygen uptake efficiency slope (OUES);³ and by measuring the respiratory response to exercise ($\dot{V}E/\dot{V}CO_2$ slope).⁴

We studied 15 patients (13 males and two females); 14 had NYHA class III and one had class IV heart failure. The aetiology was ischaemic in 10 patients, valvar in two, and idiopathic in three. Mean (SD) age was 66.2 (9.6) years. Baseline ejection fraction was 23.6 (7.5)%, left ventricular end diastolic dimension was 71.9 (9.5) mm, QRS duration was 169.3 (27.6) ms, and PR interval was 226.1 (44.8) ms. Fourteen patients had left bundle branch block and one had a pre-existing standard dual chamber pacemaker.

QRS duration was reduced to 148.1 (13.5) ms after atrioventricular pacing. The reduction in QRS duration was 21.1 (22.4) ms.

All 15 patients completed the metabolic exercise test at baseline and at follow up. Anaerobic threshold was identifiable in all exercise tests. All patients reached a respiratory exchange ratio of ≥ 1.0 with a mean (SD) of 1.17 (0.09) at baseline. At follow up, the respiratory exchange ratio was 1.17 (0.12); only one patient had a respiratory exchange ratio of < 1.0.

The exercise results are listed in table 1. Both maximal parameters and two out of three submaximal parameters improved after pacing. There was no significant correlation between baseline QRS or QRS width reduction and any of the parameters.

Maximal exercise capacity as assessed by peak $\dot{V}O_2$ is a strong independent predictor in a broad range of heart failure patients. Indeed peak $\dot{V}O_2$ is widely accepted as the best single measure of prognosis in ambulatory patients with severe heart failure.⁵ However, peak $\dot{V}O_2$ is poorly correlated with everyday physical activity which is largely a series of submaximal exercises. This may explain why there is a poor correlation between self reported exertional symptoms and maximal exercise capacity. In this regard, submaximal exercise parameters may provide better estimation of functional capacity of daily living.

Oxygen consumption at anaerobic threshold ($\dot{V}O_{2@AT}$) has been used as an estimate of submaximal capacity in heart failure patients.² There was a small increase in this variable from 10.0 (1.8) ml/min/kg before to 10.8 (1.6) ml/min/kg with cardiac resynchronisation, although this did not reach significance (p = 0.13). However $\dot{V}O_{2@AT}$ has recognised limitations.²

The kinetics of oxygen consumption at the start and end of exercise are delayed in patients with heart failure. Therefore an index of oxygen kinetics before anaerobic threshold might better reflect submaximal exercise capacity and thus patient's exertional symptoms. The OUES is one such index and was first proposed and validated as a new index of submaximal cardiorespiratory reserve by Baba and colleagues in 1996.³ Also, OUES is effort independent and thus less likely to be affected by subjective factors including training effects or intra-observer variability. For these reasons, OUES may be the most valid assessment of submaximal exercise capacity. In this study OUES showed a major improvement from 517.6 (148.2) at baseline to 612.0 (210.2) with cardiac resynchronisation (p = 0.02).

Respiratory efficiency ($\dot{V}E/\dot{V}CO_2$ slope) is also a measure of submaximal exercise capacity. Heart failure patients often have an excessive ventilatory response to exercise⁴ and this can be quantified by analysing the relation between carbon dioxide output ($\dot{V}CO_2$) and minute ventilation ($\dot{V}E$). In our study there was a clear and significant improvement in this variable with cardiac resynchronisation (41.8 (7.1) before v 38.1 (9.0) after, p = 0.04).

In conclusion, this is the first detailed analysis of the effects of cardiac resynchronisation on exercise performance and our results show that atrioventricular pacing significantly improved maximal and submaximal exercise capacity. The study has significant limitations in being small and uncontrolled; however, larger randomised studies are underway to investigate whether these

positive effects of cardiac resynchronisation on exercise performance are real and can be translated into an impact on mortality.

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Reciprocal increase of circulating interleukin-10 and interleukin-6 in patients with acute myocardial infarction

Increased circulating interleukin (IL)-10 is reported in patients with acute myocardial infarction (AMI)¹ and in mice with myocardial ischaemia/reperfusion²; induction of IL-10 mRNA in ischaemic/reperfused myocardium³ was also recently reported. Ischaemic/reperfused myocardium is protected by IL-10 through inhibition of tumour necrosis factor α production² and down regulation of IL-6 mRNA in a canine model.³ We have already shown induction of circulating IL-6, and a positive relation between natriuretic peptides in AMI.⁴ We also showed IL-6 expression in ischaemic myocardium⁵; however, no study has investigated circulating concentrations of IL-6 and IL-10 at the same time. Increased IL-10 inhibited IL-12 induction in an animal model,³ while circulating IL-12 has not been investigated in patients with AMI. In this study we show a reciprocal increase in circulating IL-10 and IL-6, but not IL-12, in patients with AMI.

Twenty patients admitted to Fujioka Hospital within six hours of the onset of AMI symptoms, and 20 healthy controls, were enrolled after informed consent was obtained. In patients with AMI, informed consent was obtained at admission; all patients immediately underwent percutaneous transluminal coronary angioplasty (PTCA). The 20 patients comprised 16 men and 4 women aged 45-69 years, and the 20 controls were 16 men and 4 women aged 45-65 years. No subjects had collagen diseases, liver disease, renal failure, malignancy, infection, autoimmune disease, or thyroid disease. The diagnosis of AMI was based on chest pain resistant to glyceryl trinitrate, electrocardiographic ST segment elevations in more than two leads with or without Q wave formation, and significant increases in plasma creatine phosphokinase

(CK) and lactate dehydrogenase (LDH). The MB isoenzyme of CK, a relatively specific indicator of myocardial damage, was elevated by > 5% in all 20 patients. Blood specimens were drawn from the femoral vein on admission and every 12 hours until 120 hours after admission. Plasma was centrifuged for 15 minutes at 3000 rpm at 4°C and stored at -80°C until assayed. IL-6 was measured with a chemiluminescent enzyme immunoassay kit (Fujirebio, Tokyo, Japan). IL-10 and IL-12 were measured by another chemiluminescent enzyme immunoassay kit (BioSource International Inc, Camarillo, California, USA). CK and LDH were measured by autoanalyser. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were measured by radioimmunoassay. Data are presented as mean (SD). Statistical analysis was performed using unpaired *t* test, analysis of variance (ANOVA), or linear regression. A probability level of $p < 0.05$ was considered significant.

Mean (SD) concentrations of IL-6, IL-10, and IL-12 in controls were 0.7 (0.5), 3.4 (1.3), and 53.9 (16.1) pg/ml, respectively, and in patients with AMI were 18.5 (8.5), 14.9 (6.2), and 55.3 (14.9) pg/ml, respectively. Mean concentrations of plasma IL-6 and IL-10 were significantly increased in patients with AMI compared with controls, and IL-10 reached a peak concentration within 24 hours after onset of chest pain (fig 1A). A prolonged increase in IL-6 concentration was detected, showing two peaks after IL-10 decreased (fig 1B). Circulating IL-12 did not differ from controls throughout the study (fig 1C). Peak concentrations of IL-10 were positively correlated with both ANP and BNP ($p < 0.01$, $R^2 = 0.41$; $p < 0.01$, $R^2 = 0.36$, respectively, data not shown). The first peak of IL-6 in AMI was correlated with ANP and BNP as previously reported (data not shown).⁴ Mean concentrations of IL-10 were significantly higher in the 11 patients with severe AMI (16.8 (6.8) pg/ml, Killip class II, III, or IV, group B) than in the nine patients with uncomplicated AMI (7.4 (3.3) pg/ml, Killip class I, group A). Neither IL-10 nor IL-12 correlated with CK or LDH, while IL-6 positively correlated with CK and LDH (data not shown). No significant relation was detected between IL-6 and IL-10, between IL-6 and IL-12, and between IL-10 and IL-12 in patients with AMI.

In this study we have shown reciprocal elevation of IL-10 and IL-6 in patients with AMI. A recent study showed that IL-10 induction and down regulation of IL-6 in myocardium required reperfusion after ischaemia, while ischaemia without reperfusion induced prolonged IL-6 mRNA expression.³ In our subjects both IL-10 and IL-6 were already increased at admission and IL-6 showed two peaks after IL-10 subsidence. All 20 patients received PTCA treatment within 12 hours after onset of chest pain; however complete reperfusion was not possible. Mean concentrations of IL-10 were significantly higher in the 11 patients with severe AMI (Killip class II, III, or IV) than in the nine patients with uncomplicated AMI (Killip I). These results were consistent with a previous report.¹ We have also shown that IL-10 was positively associated with ANP and BNP, as well as IL-6.⁴ These findings suggest that IL-10 is a marker of myocardial damage or reperfused myocardium in AMI.

In patients with AMI plasma IL-12 concentration was the same as in normal controls, in agreement with a canine model,³ and no IL-12 elevation was detected after

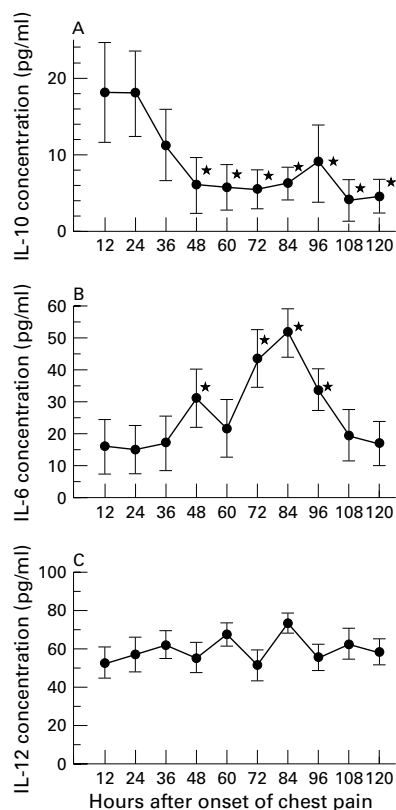


Figure 1 Time course of interleukin (IL)-10 (A), IL-6 (B), and IL-12 (C) in 20 patients with acute myocardial infarction. Data are mean (SD). * $p < 0.01$ compared with mean value at 12 hours after onset of chest pain.

IL-10 decrease. No correlation between IL-10 and IL-12 was identified.

In conclusion, our study shows reciprocal elevation of circulating IL-10 and IL-6 following AMI. Further study is required to reveal the physiological role of IL-10 in patients with AMI.

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Contrast enhanced magnetic resonance angiography and pulmonary venous anomalies

Pathological abnormalities of the pulmonary vein are relatively rare and difficult to diagnose because of restricted echo window or dilution of contrast agent during conventional angiography.^{1,2} It is usual to distinguish between pulmonary venous stenosis and anomalous pulmonary venous connection of one or more pulmonary veins, which may be associated with other cardiac malformations. Magnetic resonance (MR) imaging offers different advantages because of the wide field of view and multi planar imaging. We report on the use of contrast enhanced magnetic resonance angiography in patients with anomalies of the pulmonary veins.

Between June 1999 and October 2000, six consecutive patients in whom anomalies of pulmonary vein were suspected and/or additional imaging data were required, underwent contrast enhanced MR angiography of the heart. Patient 1 was 8 years old, patient 2 was 13 years old, patient 3 was a neonate, patient 4 was 2 months old, patient 5 was 30 months old, and patient 6 was 10 years old. Before MR angiography, five of them had undergone two dimensional echocardiography, and three had undergone cardiac catheterisation.

All MR examinations were performed after informed consent on 1.5 Tesla imaging system (Vision, Siemens, Erlangen, Germany) without ECG triggering and breath-holding. The sequence parameters were: time of repetition 3.5 ms; time of echo 1.3 ms; matrix 170 × 250; field of view 300 × 400; one signal acquired. An injection of 0.1 mmol/kg gadopentetate dimeglumine (Dotarem, Guerbet, Villepinte, France) was intravenously administered by means of an injector (Medrad, France). This injection was previously timed by a test bolus of 1 ml of gadolinium to provide maximum contrast material concentration in the vessels of interest during acquisition of central, contrast determining portion of κ space. Acquisitions were performed in coronal and sagittal planes and timed such that the peak gadolinium concentration coincided with the sampling of the central orders of κ space. Acquisition time for three dimensional MR angiography ranged from 22–44 seconds, mean (SD) 28 (10) seconds. The study was displayed as a maximum intensity projection with three dimensional multi planar reformations and was interpreted from source and reformatted images.

All patients tolerated the MR angiography well with no adverse events to the gadolinium. In patients 1 and 2, MR angiography showed partial anomalous pulmonary venous drainage to the superior vena cava with re-routing of the right pulmonary vein to the left atrium in one of them following surgery. In patient 3, MR angiography showed hypoplasia of the right pulmonary artery on axial transverse planes, and demonstrated by coronal projections the existence of a large single pulmonary vein coursing adjacent to the right border of the heart and connected to the inferior vena cava leading to the diagnosis of scimitar syndrome (fig 1). In patient 4 with severe pulmonary hypertension, MR angiography showed all right and left lower pulmonary veins connected to the left atrium but a small signal of the left upper pulmonary vein on transverse and coronal projections. Hypoplasia of this vein was suspected and

confirmed on subsequent left upper lobectomy. In patient 5 with a past history of tetralogy of Fallot, MR angiography demonstrated an aneurysm of the left pulmonary artery (20 mm in diameter) and a resultant stenosis of the left lower pulmonary vein repaired at surgery. In patient 6 with pericardial agenesis, MR angiography showed normal connection of the pulmonary veins to the left atrium but a stenosis of the left lower pulmonary vein with flow void on cine-MR imaging probably resulting from the bulging heart.

Recently, a new strategy in MR angiography has been developed to assess the aorta and its branch vessels.³ In fact, such imaging can also be used to image the intracardiac anatomical structures or vessels⁴ similar to conventional angiography with iodinated contrast agent. This technique overcomes many of the problems that degrade conventional MR angiography by using T1 shortening effect of a dynamic paramagnetic agent such as gadolinium to achieve vessel contrast. Thus, the blood contrast is no longer flow dependent but is directly related to intravascular enhancement from gadolinium chelates and three dimensional data acquisition within the heart and vessels while imaging data are being collected.³

The major advantage of three dimensional MR angiography is to provide images of the pulmonary veins that look like the venous phase of a conventional pulmonary angiogram for clinicians.⁵ In fact, such imaging is new and can depict a three dimensional perspective of the pulmonary vein connections and course,⁴ as observed in our patients with partial anomalous venous drainage, but is usually not available with other imaging techniques. Reconstruction in the three dimensions facilitates delineation of such complex anatomy. It is clear that three dimensional MR angiography appears more attractive for clinicians than the in-plane view with classical MR sequences or the projection data obtained from conventional angiography. This could be helpful to plan subsequent surgical correction as observed in the two patients

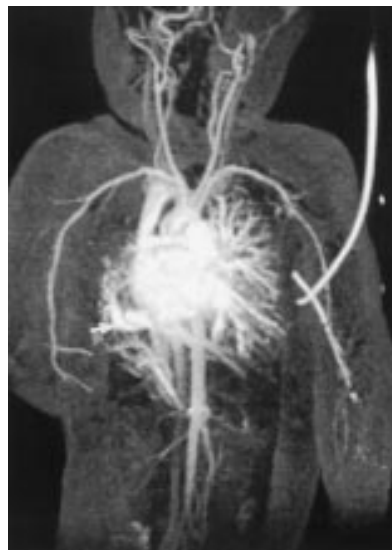


Figure 1 Patient with scimitar syndrome. Maximum intensity projection of three dimensional gadolinium enhanced magnetic resonance angiography (coronal view) reveals one single right pulmonary vein coursing adjacent to the right border of the heart and draining into the inferior vena cava (arrow).

with stenosis/hypoplasia of the pulmonary vein. A second advantage of MR angiography is its short duration, which is clearly less than that of the conventional spin echo technique, allowing its use in infants and young children. In fact the possibility to image such complex anomalies in about 30 seconds without breath-holding is clearly an advantage for use in young children. Other advantages include absence of ionising radiation, safety profile of the paramagnetic contrast agent, and lack of operator dependence.^{1,3,4}

The main limitation of this technique is related to the partial sensitivity and blurring from respiratory and cardiac motion. In fact, image quality is acceptable in most patients and such degradation is mainly related to the absence of breath-holding during data acquisition.^{1,4} The answer is to perform MR angiography with general anaesthesia in young children and/or to combine it with navigator echo technique.

This study shows that contrast enhanced MR angiography provides an excellent visualisation and three dimensional perspective of pulmonary vein anatomy. Therefore, MR angiography should be used with increasing frequency, even in infancy, as a method to evaluate suspected cases of pulmonary venous anomaly, especially when echocardiography has failed to demonstrate them, while avoiding the need for conventional angiography.

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Percutaneous aortic valve replacement: will we get there?

Percutaneous implantation of a biological valve in the pulmonary position has recently enlarged the field of interventional cardiovascular catheterisation.¹ Aortic valve disease is much more frequent than pulmonary valve disease and therefore a non-surgical approach for aortic valve replacement would be attractive. The percutaneous approach is obviously difficult because of the proximity of the aortic valve to the coronary orifices. We set out to develop a stent for valve implantation which avoids obstructing the

coronary orifices. We describe here our initial experience using the new device for percutaneous valve replacement of the aortic valve in an animal study.

A newly designed stent with a deployment strategy in two steps was developed. The first step assures the orientation of the stent in the aortic orifice. The second acts as a support for the implantable valve. A naturally valved bovine jugular vein segment was mounted as previously described inside this stent (Numed, Osypka).² The venous wall was then reduced along the commissures in order to remove the wall in front of the coronary orifices (fig 1).

The valved stent was first reduced on the outer balloon of the delivery system. The stent was then percutaneously inserted into a lamb through the right carotid onto a previously positioned guide wire. In the left ventricle, the valved stent was uncovered deploying the outer part of the stent. The system was thereafter pulled back in the area of the native aortic valve. The self centering mechanism allowed alignment of the commissures of the native valve with the commissures of the valve to be implanted. After successful centering, the balloons were successively inflated deploying the valved stent to the final diameter of 22 mm. The balloons were deflated and the delivery system was carefully retrieved, leaving the stent in position.

Transoesophageal echocardiography, and haemodynamic and angiographic evaluations were performed before and after the implantation and at the two week follow up. Data obtained confirmed the perfect function of the implanted valve and the absence of

coronary artery obstruction (fig 2). There was no complication at clinical follow up after four weeks. During the entire study, the lamb was treated according to European regulations for animal experimentation.³

Initially, we wrongly thought that the venous wall was necessary for the valve to remain competent. Our in vitro and in vivo testing showed that its removal along the commissures did not alter the function of the valve. This new valved stent design theoretically allows an orthotopic valve replacement but its precise placement is delicate. The valved stent must be precisely placed in height. An implantation above the valve near to the coronary orifices is not suitable for their perfusion. An implantation below the coronary arteries, encroaching upon the left ventricle, can impair the function of the mitral valve and enhance the risk of paraprosthetic insufficiency. The high pressures in the left system complicate the situation by increasing the risk of embolisation in the ascending aorta during the inflation of the balloon catheter or in the left ventricle at the closure of the implanted valve. This emphasises the need to anchor the stent in the precise position. We resolved these problems with the new design of our stent.

The device as it is presently designed does not allow multiple attempts before definitive deployment. One can imagine what could happen if the device obstructed the coronary arteries. The hooks reduce the risk of malpositioning but their safety must be confirmed. In humans transoesophageal echocardiography could help to position the device during the procedure. The reproducibility of the technique needs to be proven before clinical

application. Finally, the long term function of our venous valve is not yet established.

Both aortic regurgitation and aortic stenosis can potentially be treated using this technique. In the indication of aortic insufficiency, a possible limitation would be the size of the aortic root usually dilated. In aortic stenosis the calcifications and potential calcium emboli during dilatation are a major concern. The size of the delivery system might require balloon predilatation. The implantation of a stent thereafter is likely to improve the results of the balloon valvuloplasty in terms of valve area. Presumably this could improve the long term results.

Who should be the first patients to benefit from this new technique? Patients who require aortic valve replacement and who are at the highest risk of potential complications of mechanical valves are likely to primary candidates. These would include patients with high operative risks and patients who have contraindications to anticoagulation. For example, it could be a temporary solution for young women with aortic valve disease who wish to become pregnant.

The anatomy of the aortic area in lambs is very similar to humans, providing hope for the use of such a device in humans in the future. We have implanted a 22 mm venous valve which when fitted on the delivery system measured 20 French. Bigger valves are available and would not dramatically increase the size of the whole system, making percutaneous aortic valve replacement in the human possible.

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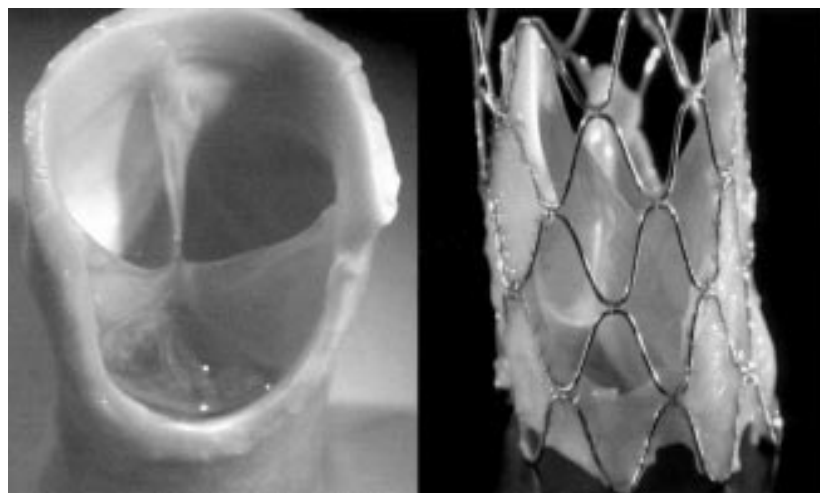


Figure 1 Left: venous valve before its suture in the supporting structure. Right: valve mounted in the platinum stent.

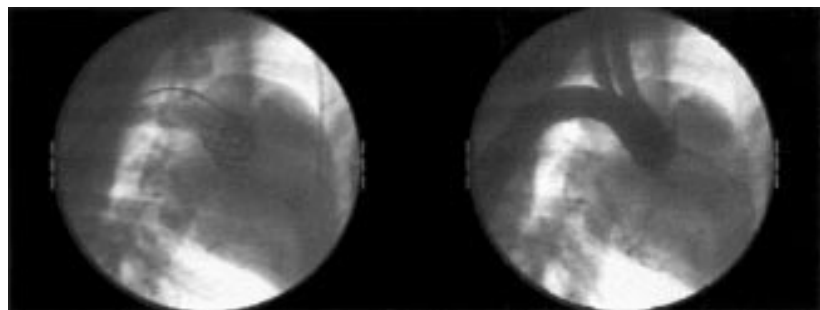


Figure 2 Left: aortic and ventricular view before angiography. Right: aortic angiography showing normal coronary arteries and competence of the implanted valve.

Tetrahydrobiopterin attenuates cholesterol induced coronary hyperreactivity to endothelin

The vascular endothelium has emerged as a critical determinant of cardiovascular health and disease. Through the release of endothelium derived contracting and relaxing factors, the endothelium modulates vascular tone/reactivity acutely, and in the long term influences the process of vascular remodelling and mitogenesis.¹ Hypercholesterolaemia is an important risk factor for coronary endothelial dysfunction. Although a variety of mechanisms have been proposed to link raised cholesterol to diminished endothelial function, recent studies suggest that hypercholesterolaemia may impair vascular function through

augmenting the actions of the potent vasoactive peptide endothelin-1.² Endothelin-1 elicits potent and protracted vasoconstriction; this effect is mediated directly via interaction with endothelin-A and endothelin-B receptors and indirectly via quenching of nitric oxide. Diminished production/availability of nitric oxide is a universal finding in patients with cardiovascular disease.

Tetrahydrobiopterin (BH4) is a cofactor for various enzymatic processes and has been implicated in the pathogenesis of hyperphenylalaninaemia, neurological disorders, Alzheimer's disease, depression, Parkinson's disease, autism, and recently cardiovascular pathology and endothelial dysfunction.³ The balance of published information suggests that in the endothelial cell, BH4 is a critical cofactor for nitric oxide synthase activation, and hence nitric oxide production is dependent upon the presence of adequate amounts of this cofactor. BH4 exerts this action through serving as an electron donor for the hydroxylation of L-arginine. Diminished concentrations of this cofactor, as observed in states of cardiovascular disease, led to an uncoupling of endothelial nitric oxide synthase with diminished nitric oxide and

exaggerated superoxide anion production.³ BH4 has emerged as an important target for pharmacological manipulation, and many studies have shown the beneficial effects of BH4 supplementation on endothelial function in vitro and in vivo.

In the present study we hypothesised that acute BH4 treatment may serve to prevent cholesterol induced hyperreactivity to endothelin-1 in vitro in the coronary vasculature. To this aim we studied the effects of the soluble cholesterol derivative, polyoxyethanyl-cholesteryl sebacate (PCS) on endothelin-1 mediated contraction and superoxide production in porcine coronary arteries in the presence and absence of BH4.

Vascular segments of porcine coronary arteries (left anterior descending artery, intact endothelium, n = 7) were studied using standard isometric organ bath procedures as previously described.⁴ Briefly, following equilibration (resting tension 2 g, Krebs physiological solution), isometric dose response curves to cumulative addition of endothelin-1 (10^{-10} to 3×10^{-8} M) were constructed in the absence and presence of PCS (30 μ g/ml, 10 minutes before

endothelin-1 dose, Sigma Inc). These responses were repeated in the presence of BH4 (0.1 mM, 15 minutes, dissolved in 0.5% ascorbic acid). For each concentration, a plateau was obtained before the subsequent dose was added. At the end of the experiment, the tissues were removed, blotted dry, and the cross sectional area of each vascular ring was calculated: cross sectional area (mm^2) = weight (mg)/(length (mm) \times density (mg/mm^3)). The density of the vascular smooth muscle was assumed to be 1.05 mg/mm^3 . The absolute tension generated was corrected for cross sectional area and expressed as g/mm^2 .

In a separate study, we examined the effects of endothelin-1, PCS, and BH4 on superoxide anion production in segments of porcine coronary arteries using lucigenin chemiluminescence as described previously.⁵ Segments of coronary arteries were incubated in Krebs-HEPES buffer for 90 minutes under the following conditions (n = 6 per group): (1) control; (2) control + BH4 (0.1 mM in 0.5% ascorbic acid); (3) control + endothelin-1 (2 nM); (4) control + PCS; (5) control + PCS + endothelin; and (6) control + BH4 + PCS + endothelin-1. BH4 was used as a pretreatment; coronary arteries were incubated with BH4 10 minutes before the additions of other interventions. The segments were transferred to the scintillation vials containing 0.25 mM lucigenin in a final volume of 2 ml.⁵ Data are expressed as counts per second per milligram of tissue (dried weight). Statistical analysis was performed using a one way analysis of variance (ANOVA) followed by a Newman Keul's test. A probability value of $p < 0.05$ was considered significant between groups.

Endothelin-1 caused a concentration dependent increase in coronary vascular tone (fig 1). Endothelin-1 mediated vasoconstriction was augmented in the presence of the soluble cholesterol derivative. Strikingly, PCS induced coronary hyperreactivity to endothelin-1 was attenuated by co-incubation with BH4 (fig 1). Although BH4 per se caused a significant decrease in endothelin-1 mediated vasoconstriction, the percent reduction of endothelin-1 responses were greater when BH4 was employed in the presence of PCS (40% v 27.9%, $p < 0.05$).

Figure 2 depicts the effects of the endothelin-1, PCS, and BH4 on superoxide anion production. Treatment with endothelin-1 alone (2 nM) increased superoxide anion production. This effect was greatly exaggerated in the presence of PCS and attenuated by co-incubation with BH4.

The key observations of the present study are: (1) the coronary vasoconstrictor effects of endothelin-1 are exaggerated by acute in vitro administration of PCS; (2) co-incubation of porcine coronary arteries with the nitric oxide synthase co-factor BH4 attenuates the effects of PCS on endothelin-1 induced vasoactivity; and (3) the production of superoxide by coronary arteries exposed to PCS + endothelin-1 is greater than each intervention alone. Importantly, this response is attenuated by BH4 supplementation. These data uncover for the first time the functional and acute interaction between cholesterol, endothelin-1, and BH4 in modulating coronary vascular tone.

The synergistic effects of cholesterol on endothelin-1 induced contraction were recently reported in isolated rat aortae.² The

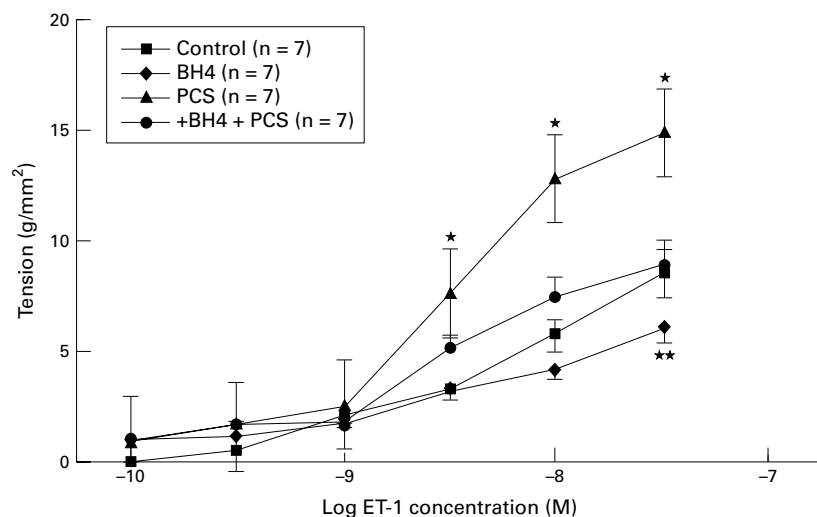


Figure 1 Dose response curve to endothelin-1 in porcine coronary arteries in the presence and absence of tetrahydrobiopterin (BH4, 0.1 mM) and the soluble cholesterol derived, polyoxyethanyl-cholesteryl sebacate (PCS, 30 μ g/ml). Data are expressed as developed tension (g) corrected for cross sectional area (mm^2). In the presence of PCS, endothelin-1 mediated vasoconstriction was enhanced; this response is attenuated by co-incubation with BH4. Although BH4 alone attenuated endothelin-1 responses the degree of attenuation in the presence of PCS was greater (40% v 27.9%, $p < 0.05$). * $p < 0.05$ PCS different from control, BH4, and BH4 + PCS. ** $p < 0.05$, BH4 different from control.

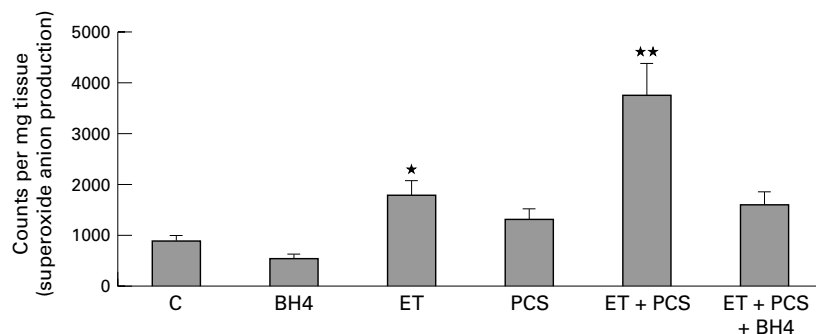


Figure 2 Superoxide anion production expressed as counts per mg tissue in porcine coronary arteries exposed to endothelin-1 (2 nM), PCS (30 μ g/ml), and BH4 (0.1 mM). Endothelin-1 increased superoxide anion production by coronary arteries. This response was exaggerated by PCS and attenuated by BH4. Superoxide anion was measured in isolated vascular segments by lucigenin chemiluminescence in the presence of 10 mM diethyldithiocarbamate. * $p < 0.05$, different from control, BH4, PCS and endothelin (ET) + PCS + BH4. ** $p < 0.05$, different from all groups.

authors suggest that this may be mediated through a mitogen activated protein kinase and cyclooxygenase-2 dependent mechanism. The present study extends these observations to the coronary circulation and suggests that diminished production and/or availability of BH4 may be one of the mechanisms mediating the effects of PSC on ET-1 action. Diminished concentrations of BH4 leads to the uncoupling of nitric oxide synthase with the resultant production of superoxide anion and nitric oxide quenching. Supplementation of BH4 may restore endothelial function by preventing the uncoupling of nitric oxide synthase and facilitating the flow of electrons from L-arginine to nitric oxide. Although hypercholesterolaemia and endothelin-1 independently contribute to coronary endothelial dysfunction, we suggest that the synergistic effects of this combination

may be ascribed, in part, to diminished availability of BH4. These data are important since patients with raised cholesterol often have coexistent elevations in endothelin-1.⁶

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