INTERVENTIONAL CARDIOLOGY AND SURGERY

Comparison of the effect of enalapril and losartan in conjunction with surgical coronary revascularisation versus revascularisation alone on systemic endothelial function

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Objectives: To investigate the effect of enalapril, losartan, and surgical coronary revascularisation on endothelial function, and the role of the angiotensin converting enzyme (ACE) insertion (I)/deletion (D) polymorphism.

Design: Randomised, controlled, blinded end point study.

Setting: University tertiary referral cardiac centre.

Patients and interventions: 49 men awaiting coronary artery bypass grafting (CABG) were randomly assigned to treatment with losartan, enalapril, or control for two months before and three months after surgery.

Main outcome measures: Endothelial function was blindly analysed by brachial artery flow mediated dilatation (FMD) and ACE I/D genotype was determined.

Results: FMD was impaired at baseline (1.0–1.7%) and after five months had improved to 5.2% with enalapril ($p = 0.015$), 5.0% with losartan ($p = 0.0004$), and 3.0% with CABG alone ($p = 0.05$). Patients with the II genotype had lower baseline FMD than those with DI or DD (0.1% v 1.7%, $p = 0.038$) and after enalapril or losartan treatment had greater improvement in FMD (mean (SEM) 7.1 (1.1)% than patients with DI (3.1 (1.3)%, $p = 0.024$) or DD genotype (3.1 (1.1)%, $p = 0.02$).

Conclusions: Enalapril and losartan, with surgical coronary revascularisation, significantly improve systemic endothelial function. Revascularisation alone produces a quantitatively smaller, but still significant, improvement. The ACE genotype significantly modulates this response. Patients with the II genotype have a more pronounced impairment in endothelial function at baseline and a greater improvement in response to treatment with these agents.

The endothelium is thought to play a key role in the pathogenesis of atherosclerosis,¹ a role in which angiotensin II appears also to have an important influence.² Endothelial vasomotor function provides an easily measured assessment of endothelial function and prognostically stratifies patients with risk factors for, or evidence of, coronary artery disease.^{3,4}

Angiotensin converting enzyme (ACE) inhibitors appear to have important effects on reversing endothelial vasomotor dysfunction in several pathological conditions.^{5–11} The angiotensin II type 1 (AT1) receptor antagonist losartan has also been shown to improve endothelial function in both coronary artery disease and diabetes,^{8,12,13} although this has not been confirmed by all investigators.⁹ Furthermore, the effect of the insertion (I)/deletion (D) polymorphism in intron 16 of the ACE gene on endothelial function remains controversial, with both the D and I alleles being associated with a greater improvement in endothelial function after ACE inhibition.^{9,11}

The effect of coronary revascularisation on systemic endothelial function in humans has not been investigated. In animal models, the presence of endothelial dysfunction during ischaemia–reperfusion has been documented,¹⁴ and ischaemia and reperfusion attenuate nitric oxide release and nitric oxide synthase activity.¹⁵ In humans, ischaemia has been shown to produce adverse alterations in vasoactive substances both in the coronary circulation¹⁶ and systemically.^{17,18} The relief of myocardial ischaemia may therefore produce both local and systemic alterations in endothelial function.

We therefore investigated the effect of ACE inhibition versus AT1 receptor antagonism on endothelial function in

patients with coronary artery disease awaiting coronary artery bypass grafting (CABG). The effect of these agents before and after surgical coronary revascularisation, as well as the effect of revascularisation alone, was evaluated, together with any interaction with the ACE I/D polymorphism.

METHODS

Patients

The study population comprised men with normal left ventricular function (ejection fraction > 60% on left ventricular angiography) and stable angina awaiting CABG who had not received an ACE inhibitor, AT1 receptor antagonist, or β blocker during the preceding three months. Exclusion criteria were systolic blood pressure < 100 mm Hg before randomisation, known or suspected renal artery stenosis, type 1 diabetes or poorly controlled (haemoglobin A_{1c} > 6.5%) type 2 diabetes, stroke, or transient ischaemic attack in the previous three months, myocardial infarction within the past six months, previous CABG, or valvar heart disease requiring corrective surgery. Patients were randomly assigned to treatment with enalapril (10 mg twice daily) or losartan (50 mg once daily) or control (no adjustment to treatment) two months before surgery. The study was a prospective, randomised, open label treatment, blinded end point design. The study was conducted in accordance with the Declaration

Abbreviations: ACE, angiotensin converting enzyme; AT1, angiotensin II type 1 receptor; CABG, coronary artery bypass grafting; D, deletion; FMD, flow mediated dilatation; I, insertion

of Helsinki and was approved by the local research ethics committee. All patients gave informed consent.

Endothelial function

Endothelial function was examined at randomisation, on the day before surgery (after two months of study drug treatment), and three months after CABG by a non-invasive technique with high resolution external ultrasound to measure endothelial dependent flow mediated dilatation (FMD) of the brachial artery. Results were analysed in a blinded fashion. The stimulus for FMD is provided by reactive hyperaemia with a cuff on the lower arm inflated to suprasystolic pressure for five minutes while glyceryl trinitrate is used as an endothelium independent vasodilator.¹⁹ Scans were recorded on a GE Vingmed System V ultrasound machine with a 5–10 MHz flat linear array probe (GE Ultrasound Europe, Bedford, UK). Scanning was performed in a temperature controlled environment (22°C), with patients fasted and having avoided cigarettes, alcohol, or caffeine for 12 hours. All medications were omitted for > 24 hours before the study with the exception of trial medication. Images were recorded on S-VHS tape and analysed off line with the observer blinded to patient status. Brachial artery diameters were calculated from the average of four readings by an edge detection software package (Dynamic Endothelial Assessment Program, CDMED, Montreal, Canada). This technique is highly reproducible in our laboratory with absolute interobserver and intraobserver variabilities in FMD of < 1%.

ACE I/D genotyping protocol

The I/D mutation sequence in intron 16 of the ACE gene was amplified by polymerase chain reaction, with sense and antisense primers (5'-GGA GAG AGA CTC AAG CAC GC-3', 5'-TTG ATG AGT TCC ACG TAT TTC G-3') and an insertion specific primer (5'-TGG GAT TAC AGG CGT GAT ACA G-3'). Polymerase chain reaction was undertaken with 0.2–0.5 µg of genomic DNA in a 25 µl reaction mixture containing 10 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 200 µmol/l each of dATP, dCTP, dGTP, and dTTP, 1.5 mmol/l MgCl₂, 25 ng of each primer, and 0.5 units of Taq DNA polymerase. The amplification cycle was performed on a Techne GeneE Thermal Cycler (Techne, Duxford, Cambridge, UK) and entailed 1.5 minutes' denaturation at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and one minute at 72°C. This was followed by 10 minutes' extension at 72°C. The product was stained with ethidium bromide at a concentration of 0.5 µg/ml and separated by electrophoresis on a 2% agarose gel. All genotyping was conducted in duplicate by observers blinded to the clinical status of the patient, with the use of positive and negative controls.

Surgery

CABG was performed with midline sternotomy incision, intermittent cross clamp fibrillation, normothermic cardiopulmonary bypass, and a standardised anaesthetic. All patients underwent full revascularisation with the formation of left internal mammary artery graft to the left anterior descending artery and additional venous or arterial grafts to the rest of the coronary anatomy as indicated by distribution of the coronary disease and the clinical opinion of the surgeon.

Statistical analysis

All data were analysed with SPSS for Windows version 10.0 (SPSS Inc, Chicago, Illinois, USA). Categorical variables were analysed with χ^2 Fisher's exact test. Continuous variables with a normal distribution were analysed by Student's *t* test, without assuming equal population variances, for analyses

between two groups and by analysis of variance for analyses between more than two groups. Continuous variables with a non-normal distribution were analysed by the Mann-Whitney U test for two groups and by the Kruskal-Wallis H test for more than two groups. All analyses were two tailed and *p* < 0.05 was considered significant. Data are presented as mean (SEM).

Power calculation

On the basis of previously published data for the effect of ACE inhibitors on endothelial function,¹⁰ this study was designed to have a > 90% power of detecting an effect on endothelial function by either of the treatments at *p* < 0.05.

RESULTS

Forty nine patients were enrolled and were well matched with respect to baseline characteristics (table 1). All patients reached the target dose in both treatment groups and a pre-operative tablet count showed that the cohort was 99.6% compliant with treatment. There were two perioperative deaths (enalapril and losartan treated patients) and a further two patients (losartan treated) had graft occlusion, reoperation, and Q wave myocardial infarctions. In view of the possible impact of this complication on endothelial function, FMD was analysed after CABG both including and excluding these patients.

Figure 1 shows the effect of treatment with enalapril and losartan versus surgical revascularisation alone on endothelial function. At baseline, there was no significant difference in FMD between the groups, with all groups having impaired FMD responses of 1.0–1.7%. After two months of treatment, FMD was not significantly altered (1.3–2.6%), although enalapril and losartan treated patients showed trends towards improvement. After five months of treatment and three months after surgical revascularisation, FMD had increased in all groups, with significant changes compared with baseline in the enalapril group to 5.2% (*p* = 0.015), in the losartan group to 5.0% (*p* = 0.0004), and in the revascularisation only group to 3.0% (*p* = 0.05).

FMD at five months did not differ between enalapril and losartan treatment, either in the whole cohort (5.2% *v* 5.0%, not significant) or when ACE genotypes were examined. Although FMD at five months increased to a similar degree in patients treated with enalapril or losartan and was greater in both treatment groups than in the control group, this comparison was only of borderline significance between losartan and controls (*p* = 0.058). If the data from the two losartan treated patients who had perioperative Q wave myocardial infarctions are excluded from the postoperative analysis, FMD at five months in this group increases to 5.2% (*p* = 0.001 *v* baseline; *p* = 0.042 *v* controls). Brachial artery Doppler measurements indicated that hyperaemia of > 300% was achieved in response to ischaemia in all groups at all time points, and baseline brachial artery diameter did not vary between groups. Endothelium independent dilatation in response to sublingual glyceryl trinitrate was maintained at all times, being 10–20%.

Table 2 shows frequencies of the ACE I/D polymorphism, which were in Hardy-Weinberg equilibrium (one patient could not be genotyped despite repeated attempts). Examination of preoperative and postoperative endothelial function by ACE genotype (fig 2) showed that at baseline patients with the DD (1.2 (0.6%)) and DI (2.0 (0.5%)) genotypes had greater FMD than those with the II genotype (0.1 (0.8%)) (DD+DI *v* II, *p* = 0.038). Subsequently, however, this relation was not observed, with preoperative and postoperative FMD similar responses in all groups regardless of ACE genotype.

Table 1 Baseline patient characteristics

	Control (n = 15)	Enalapril (n = 16)	Losartan (n = 18)	p Value
Age (years)	63.6 (2.2)	64.8 (1.6)	62.9 (1.7)	0.7
Hypertension	8 (53.3%)	6 (37.5%)	9 (50.0%)	0.6
Prior myocardial infarction	4 (26.7%)	8 (50.0%)	10 (55.6%)	0.2
Prior stroke/TIA	0	2 (12.5%)	1 (5.6%)	0.3
Type 2 diabetes	2 (13.3%)	2 (12.5%)	1 (5.6%)	0.7
Current smoker	0 (0%)	2 (12.5%)	0 (0%)	0.1
Ejection fraction (%)	76.1 (2.3)	71.5 (2.7)	74.7 (1.6)	0.4
Total cholesterol (mmol/l)	4.6 (0.15)	5.0 (0.23)	4.5 (0.16)	0.2
HDL cholesterol (mmol/l)	1.15 (0.09)	1.17 (0.07)	1.21 (0.06)	0.9
Triglycerides (mmol/l)	1.9 (0.35)	1.9 (0.22)	1.8 (0.18)	0.8
Systolic BP (mm Hg)	138.8 (5.3)	144.3 (4.2)	141.7 (2.9)	0.7
Diastolic BP (mm Hg)	82.9 (2.3)	81.5 (2.5)	81.6 (2.1)	0.9
Creatinine (μ mol/l)	110.7 (5.6)	102.3 (3.5)	103.2 (2.4)	0.5
Body mass index (kg/m ²)	27.9 (0.9)	28.0 (1.2)	27.5 (0.6)	0.9
Aspirin	14 (93.3%)	15 (93.8%)	18 (100%)	0.5
Statin	12 (80.0%)	15 (93.8%)	15 (83.3%)	0.5
Ca ²⁺ channel antagonist	14 (93.3%)	12 (75.0%)	16 (88.9%)	0.3
Nitrate	11 (73.3%)	11 (68.8%)	11 (61.1%)	0.7

Data are mean (SEM) or number (%).

BP, blood pressure; HDL, high density lipoprotein; TIA, transient ischaemic attack.

As this relation of FMD to ACE genotype may be skewed at these subsequent time points by the greater improvement in FMD observed in patients treated with enalapril or losartan, the response to treatment at two (before the operation) and five (after the operation) months was also examined in these treated patients alone (fig 3). It can be seen that patients with the II genotype had lower FMD at baseline (DD+DI ν II, $p = 0.016$). After two months of treatment, FMD had improved in all groups, with no significant differences in FMD between them. By five months FMD had significantly improved in all groups, with a graded response noted among the genotypes: DD 4.4 (1.1)% ($p = 0.018 \nu$ baseline), DI 5.5 (1.1)% ($p = 0.03 \nu$ baseline), II 6.6 (1.2)% ($p = 0.005 \nu$ baseline). The degree of improvement observed after active drug treatment was greatest in patients with the II genotype (7.1 (1.1)%) and was significantly higher than in patients

with the DI (3.1 (1.3)%, $p = 0.024$) or DD genotype (3.1 (1.1)%, $p = 0.02$).

DISCUSSION

We have shown that both losartan and enalapril, in combination with surgical coronary revascularisation, significantly improved systemic endothelial function, whereas revascularisation alone produced a quantitatively smaller, but still significant, improvement. Furthermore, the ACE genotype significantly modulated this response, with patients with the II genotype having a more pronounced impairment in endothelial function at baseline and a greater improvement in response to treatment with drugs antagonising the renin-angiotensin system.

Effect of enalapril and losartan on endothelial function

The improvement in endothelial function we have observed after treatment with enalapril and losartan confirms the finding of other authors studying a variety of pathological conditions including coronary artery disease, hypertension, heart failure, and diabetes treated by both invasive and non-invasive techniques.^{5-8 10-13} One study that showed no effect of enalapril or losartan on endothelial function, but a positive effect with the ACE inhibitor quinapril, differed in discontinuing the trial medication at least 72 hours before brachial artery examination.⁹ Most other investigators, including ourselves, have continued treatment up to the day before⁸ or the day of^{6 13} examination. At least some of the endothelial recovery produced by enalapril and losartan is caused by an acute effect of the drugs,^{6 12} and this prolonged drug-free period in the BANFF (brachial artery normalisation of forearm function) study may have been responsible for some of the differences observed. The authors postulate that the

Figure 1 Brachial artery flow mediated dilatation (FMD) at baseline, two months (preoperative), and five months (postoperative) according to treatment arm (enalapril, losartan, control).

Table 2 Genotype distribution of angiotensin converting enzyme (ACE) insertion (I)/deletion (D) polymorphism by treatment group

Genotype	Number	Control (n = 15)	Enalapril (n = 16)	Losartan (n = 18)
DD	15 (31%)	1	5	9
DI	24 (50%)	9	9	6
II	9 (19%)	5	2	2

Frequency distribution is in Hardy-Weinberg equilibrium.

Figure 2 Brachial artery FMD at baseline, two months (preoperative), and five months (postoperative) by angiotensin converting enzyme (ACE) insertion (I)/deletion (D) genotype in the full cohort. *Baseline FMD, DD+DI v II, $p=0.038$.

superior effect of quinapril may relate to its greater tissue specificity for ACE. Other investigators have also noted a greater effect on endothelial function of intra-arterial infusion of quinaprilat over enalaprilat,⁷ with enalaprilat in fact producing no improvement. Our findings, however, are in keeping with previous work showing a significant improvement in endothelial function after treatment with enalapril.⁶

The recovery in endothelial function noted with enalapril and losartan in our study occurred almost exclusively in the postoperative period, with very little change occurring during the two month treatment before revascularisation. This may indicate an additional, or even synergistic, effect of these agents when administered in association with CABG, possibly related to the relief of ischaemia, or a time dependent effect of such treatment. The effect of psychological stress on endothelial function in our patients who were studied one day before CABG should also be considered. Although we did not formally test subjective stress levels, impending coronary surgery is likely to induce a considerable degree of psychological stress in patients, and such factors have been shown to impair endothelial function directly.²⁰ It is possible, therefore, that any improvement in endothelial function produced by enalapril or losartan preoperatively was masked by this confounding factor.

Effect of coronary revascularisation on endothelial function

The observation that surgical coronary revascularisation alone produced an improvement in endothelial function is interesting and has, to our knowledge, not been previously reported. It is, perhaps, difficult to postulate mechanisms by which a local treatment such as CABG can produce alterations in generalised endothelial vasomotor function. None the less, it is possible that the relief of coronary ischaemia produces more widespread alterations in vasomotor tone through effects on systemic factors. Such an effect

Figure 3 Brachial artery FMD at baseline, two months (preoperative), and five months (postoperative) by ACE I/D genotype among patients treated with enalapril or losartan.

may result from alterations in the nitric oxide pathway or through other vasoactive substances.

A clear relation exists between endothelial dysfunction and ischaemia-reperfusion in both animal models^{14,21} and humans after myocardial infarction in whom endothelial dysfunction is seen in the infarct related artery, an effect that improves with time.²² Nitric oxide appears to be involved in these responses, with both brief and prolonged episodes of ischaemia being associated with reductions in nitric oxide metabolites in cardiac interstitial fluid,²³ and with the activity of nitric oxide synthase and basal nitric oxide release being reduced by ischaemia and reperfusion.¹⁵ The relief of myocardial ischaemia may therefore beneficially affect local nitric oxide metabolism.

Ischaemia may also lead to alterations in the production of other vasoactive substances. Unstable angina, and pacing to produce angina, in humans has been shown to produce increases in the vasoconstrictor thromboxane A2 in the coronary venous circulation.¹⁶ Reduced systemic concentrations of prostacyclin have been found in patients with angina compared with controls, with further reductions in those patients with chest pain at rest.²⁴ Furthermore, myocardial ischaemia is associated with systemic increases in the vasoconstrictors neuropeptide Y¹⁷ and endothelin.¹⁸ We postulate that coronary grafting, by reducing ischaemia, may produce both local and systemic alterations in vasoactive substances, including nitric oxide, thus improving coronary and more generalised endothelial function.

Effect of ACE genotype on endothelial function

Our finding that recovery of endothelial function in response to ACE inhibition and AT1 receptor antagonism was greatest in patients with the II genotype tends to support previous work that found the beneficial effect of quinapril to be confined to patients with the I allele.⁹ However, other investigators have reported a greater improvement in endothelial function after acute ACE inhibition only in patients with the D allele.¹¹ Increased ACE activity associated with the D allele has been postulated as an explanation both

for the lack of improvement noted in patients with the DD genotype,⁹ in whom ACE inhibition may be less efficacious, and for the greater improvement seen with the same genotype,¹¹ due to the potential for a proportionately greater effect from ACE inhibition. Support for the former hypothesis is found in data showing that the degree and duration of attenuation of pressor response with enalapril is greater in patients with the II genotype²⁵ and in work with other organ systems showing attenuation of the physiological effects of ACE inhibition with the D allele.²⁶ This is an area requiring further study.

Our observation regarding the effect of the ACE I/D polymorphism on endothelial function at baseline is novel but should be viewed with caution, given the limited size of our cohort and that it is difficult to explain within the current understanding of the effect of this polymorphism on renin-angiotensin system metabolism. Previous studies have produced conflicting results, with some noting no effect of this polymorphism on baseline endothelial function in patients without coronary risk factors,²⁷ in normotensive patients,²⁸ or in patients with coronary artery disease or risk factors.⁹ Other studies have shown a blunting of endothelial function with the D allele in healthy people,²⁹ in hypertensive patients, but not healthy controls,³⁰ or selectively in the femoral but not brachial circulation.³¹ This effect of the D allele is again hypothesised to be caused by its association with increased ACE activity, with increased angiotensin II generation and bradykinin breakdown both adversely affecting nitric oxide bioavailability. Contradictory findings have been postulated to be caused by differences in the populations studied, the techniques used (invasive versus non-invasive), or the site of examination. The reasons behind our observation of a greater impairment in baseline endothelial function in patients with the II genotype with atherosclerosis are unclear, should not be viewed as conclusive, and require further evaluation in other cohorts.

Conclusion

Our study shows that the ACE inhibitor enalapril and the AT1 receptor antagonist losartan both significantly improve endothelial function in association with surgical coronary revascularisation. Furthermore, revascularisation alone produces a smaller, but still significant, improvement in systemic endothelial function. In this cohort, the II genotype was associated with impaired endothelial function at baseline and with greatest improvement in endothelial function after treatment with enalapril or losartan.

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