The effect of amlodipine on endothelial function in young adults with a strong family history of premature coronary artery disease: a randomised double blind study

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Abstract

Endothelial dysfunction, an early event in atherogenesis, has been demonstrated in young asymptomatic subjects with a strong family history of premature coronary artery disease (CAD). In these subjects, preventive measures involving risk factor modification are not appropriate, and strategies employing novel antiatherogenic agents, such as the dihydropyridine calcium channel blocker, amlodipine, may be useful. Ninety-one subjects (mean age, 28.6 years; range, 18–40) with a strong family history of premature CAD and no other identified vascular risk factors were randomised to either 5 mg amlodipine (49 subjects) or placebo (42 subjects). Brachial artery flow mediated dilatation (FMD) (endothelium-dependent response) and response to glyceryltrinitrate (GTN) (direct smooth muscle dilator) were assessed non-invasively at baseline, and after 12 and 24 weeks using high-resolution vascular ultrasound. In those treated with amlodipine, mean FMD increased from 2.32 ± 2.23% at baseline to 3.52 ± 3.1% at 24 weeks (P < 0.005). However, FMD also increased in the placebo group from 1.64 ± 2.12 to 3.37 ± 2.68% (P < 0.002), and the difference between the FMD response in the amlodipine and placebo groups was not significant. Dilatation to GTN did not change in either group. Therefore, impaired endothelial function improved in family history subjects taking both amlodipine and placebo, but there is no difference between the groups. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

A history of premature coronary artery disease (CAD) in a first-degree relative is an independent risk factor for coronary heart disease [1–5] and may convey a 2.5- to 7-fold increase in risk of death from coronary disease [6]. Although this inherited vascular risk may be mediated by genetically influenced cardiovascular risk factors, including diabetes and hyperlipidaemia [7–10], it is also likely that a variety of genes interact to alter the arterial wall and/or its susceptibility to damage from risk factors [11].

Damage to the vascular endothelium is an initiating event in experimental studies of atherogenesis [12]. This is thought to involve reduced activity of nitric oxide (NO) [13,14], a key ‘anti-atherogenic’ molecule that not only modifies vascular tone, but also plays a key role in regulating vascular permeability, platelet adhesion/aggregation, leukocyte/vessel wall interaction and smooth muscle proliferation [15], all recognised events in early atherosclerotic vascular disease.

Endothelial dysfunction has been demonstrated in young, asymptomatic, subjects who have established cardiovascular risk factors, such as smoking, hypercholesterolaemia and diabetes, and patients with established atherosclerosis [16–19]. In these preclinical subjects, experimental studies have shown that targeted risk factor modification, such as aggressive cholesterol reduction, has led to improved endothelial function.
Clinical studies involving similar interventions have demonstrated further long-term benefits in reduced cardiovascular morbidity and mortality [21]. Similarly, impaired endothelium-dependent dilatation, an early marker of endothelial dysfunction, has recently been demonstrated in the conduit arteries of young adults whose only identifiable vascular risk factor is a strong family history of CAD [22]. These individuals have no obvious modifiable risk factors and conventional preventative strategies may not be appropriate.

In established atherosclerotic disease, the potential role of calcium channel blockers (CCB) has been examined with angiographic regression/progression [23–25], and now morbidity and mortality studies [26]. Experimental evidence also suggests that CCB may play a role in disease development, but this has not been studied clinically [27]. The mechanism by which CCB exert this anti-atherogenic effect is uncertain but might involve the suppression of smooth muscle cell (SMC) proliferation and connective tissue migration in the vascular wall [28]. Amlodipine, a highly lipophilic dihydropyridine CCB, has been shown to have additional properties, inhibiting the oxygen free radicals involved in lipid peroxidation [29], stabilising cell membrane lipid bilayers [30] and preventing vascular endothelial damage in cholesterol fed primates [31]. As this agent has been widely used clinically and is well tolerated, it was felt it might represent a novel therapeutic approach applicable to family history subjects.

Therefore, the effects of the CCB amlodipine on conduit artery endothelial function was examined in a cohort of young adults whose only identifiable cardiovascular risk factor was a family history of premature CAD, to determine whether improvements of potential clinical significance could be achieved at this early stage in the natural history of atherosclerotic disease.

2. Methods

2.1. Subjects

We identified 193 patients (men ≤ 50 years, women ≤ 60 years) with premature CAD (angiographically proven ≥50% stenosis of one or more of the major epicardial coronary arteries, or with myocardial infarction chest pain, development of Q-waves on the resting electrocardiogram (ECG) and cardiac enzyme rise) from coronary care and angiography records from three London hospitals, over a 30 month period. These patients were assessed for cardiovascular risk factors with a questionnaire concerning smoking history and family history of CAD, and details of their lipid profile and history of hypertension were obtained from hospital records or their primary care physician. Those with familial hypercholesterolaemia (n = 24) or diabetes mellitus (n = 15), were excluded. The remaining 154 patients were contacted to determine whether they had any first-degree relatives aged between 18–45 years who were lifelong non-smokers, not hypertensive (resting blood pressure < 140/90) or diabetic (fasting plasma glucose < 5.2 mmol/l) and who were receiving no regular vaso-active medications. Of the 133 who replied, 89 patients had 141 first-degree relatives (86 male, 55 female) who were willing to participate.

One hundred and forty-one family history subjects attended for a preliminary visit, at which their eligibility for the study was assessed with confirmation of a strong family history of CAD (already defined), medical and drug history, and general physical and cardiac examination including supine blood pressure and a resting 12-lead electrocardiogram. Subjects then underwent a non-invasive assessment of vascular function. Ninety-eight subjects whose flow mediated dilatation is impaired (defined as a flow mediated dilatation below 1 standard deviation from the mean responses seen in 210 healthy subjects without vascular risk factors, matched for vessel size), were included in the study.

2.2. Study design

This was a randomised, placebo-controlled, double-blind parallel group study consisting of a 4-week placebo run-in phase followed by a 24-week double-blind treatment phase. At entry, subjects were randomised to receive placebo or 5 mg amlodipine. During the first 4 weeks, however, each subject received placebo so that compliance could be monitored. Endothelial function was then assessed non-invasively, at baseline, and after 12 and 24 weeks of treatment phase, using high resolution external ultrasound as previously described [32]. Subjects were instructed to continue with their normal diet and to report any adverse events, co-existing illness or medication for the duration of the study. At each visit, blood samples were taken for full blood count and biochemistry. Fasting total cholesterol and plasma triglycerides were measured at recruitment and on completion of the study using the cholesterol C-system high-performance CHOD-PAP and GPO-PAP high performance enzymatic colourimetric test, respectively (Boehringer Mannheim GmbH, Diagnostica). High-density lipoprotein (HDL)-cholesterol was measured after precipitation of apoprotein B containing lipoproteins, and low-density lipoprotein (LDL)-cholesterol calculated according to the Friedwald formula [33]. The study was approved by the local research ethics committee, and all subjects gave informed written consent.
2.3. Assessment of endothelial function

Subjects lay at rest for at least 10 min prior to the first scan and remained supine throughout the procedure. The right brachial artery was imaged in longitudinal section 2–10 cm above the elbow using a standard 7 MHz linear array transducer supported by a flexible stereotatic clamp and an Accuson 128XP/10 ultrasound system. The centre of the artery was identified when the clearest image of anterior and posterior arterial wall layers was seen. Depth and gain settings were set to optimise the lumen/arterial wall interface, and the transmit (focus) zone was set to the depth of the anterior wall in view of the greater difficulty in evaluating this interface. Machine operating parameters were not altered for the duration of the study. The image was magnified using a resolution box function and resting blood flow estimated using pulsed wave Doppler with the cursor set at 70° to the longitudinal axis of the artery and the range gate (1.5 mm) in the centre of the artery. A segment of the artery was selected for analysis and 5 s radio-frequency signal from this segment routed to an A-mode tracking device (Ingenious Systems, The Netherlands). Following careful placement of volume sample cursors, at the vessel wall interfaces, vessel wall movement was tracked and end diastolic diameter determined on a beat by beat basis with a spatial resolution of 50 μm.

Once a stable and clear image was achieved, the transducer position was fixed, and both the arm and transducer remained in the same position throughout the study. Hard-copy images of the brachial artery were taken and notes made of the transducer and arm position, enabling reproduction of conditions and measurement of the same segment of artery at subsequent visits. Resting brachial artery internal diameter was measured at end diastole for consecutive beats over a 5-s interval and the mean internal diameter calculated. Reactive hyperaemia was induced by inflating a pneumatic tourniquet placed around the forearm at a site distal to that being scanned to 300 mmHg and its rapid release after 4.5 min. The increase in brachial arterial flow over 30 s following cuff release was determined using pulsed wave Doppler as already described. Brachial artery diameter was measured 55–65 s after release of the tourniquet and flow-mediated dilatation (FMD) expressed as the percentage increase in diameter in response to reactive hyperaemia. After 10–15 min rest to allow for vessel recovery, a further resting scan was recorded. Sublingual 400 μg glyceryl trinitrate (GTN) was administered, and the response to this endothelium-independent dilator measured after 3 min. Haemodynamic parameters and ECG were monitored throughout the study.

Brachial artery FMD may be blocked by specific antagonists of NO synthase [34], and measures of FMD using this technique correlate with invasive assessments of coronary endothelial function and atherosclerosis [35]. The non-invasive technique has been shown to be accurate and reproducible over the time course of the current study [36,37].

2.4. Data analysis

All scans were recorded onto super VHS videotape. Printouts of the A-mode signal and cursor placement were made for each scan. Scans were checked for technical errors by two independent observers who remained blinded to the origin of the scan. Scans in which either the image at subsequent studies was not replicated, a satisfactory distensibility waveform was not achieved, or cursor placement was incorrect were excluded from the final analysis. Resting volumetric flow was calculated for each study by multiplying the velocity time integral corrected for angle by the heart rate and vessel cross-sectional area. This method may lead to overestimation of blood flow, although inaccuracies are consistent, allowing comparison between visits and individuals. Reactive hyperaemia was calculated as the ratio of maximal flow (determined from the pulse wave Doppler signal and heart rate) during the first 15 s after tourniquet release to that at baseline and expressed as a percentage increase in flow.

2.5. Statistical analysis

Descriptive data are expressed as mean value ± S.D. Subjects who were withdrawn after randomisation were included in the analyses up to and including the time of withdrawal (intent-to-treat population). Changes in the measures of vascular function within groups and between groups were assessed using the Wilcoxon signed rank and Wilcoxon rank sum test, respectively. Baseline disease variables were divided into three groups as follows: Group 1, age, sex; group 2, vessel size, resting volumetric flow, reactive hyperaemia, systolic blood pressure (BP), diastolic BP; group 3, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides. A simple linear regression model was used to determine the variables from each group that appeared to be most closely associated with the brachial artery ultrasound variables of FMD and GTN-induced dilatation at baseline and change in these variables over the duration of the study. These variables were then applied in a multiple regression model to explore relationships that might determine vascular reactivity at baseline and, with the addition of treatment group as an independent variable, change in vascular function over the duration of the study. Statistical significance was inferred at a P value < 0.05.
3. Results

3.1. Study subjects

After the 4-week placebo screening phase, all 98 subjects achieved satisfactory compliance (>80%) and were entered into the randomised phase of the study. Of the randomised subjects (50 amlodipine, 48 placebo), seven subjects (one amlodipine and six placebo) withdrew before receiving a single dose of study drug for personal or logistical reasons. Of the ‘intent-to-treat’ population (those in whom a vascular scan was performed) of 91 (49 amlodipine, 42 placebo), eight subjects who received amlodipine and three who received placebo were withdrawn prematurely and did not complete the study. Twenty-four amlodipine subjects and 22 placebo subjects had clinical adverse events (P = 0.75). The dose of treatment was reduced or temporarily discontinued in two amlodipine subjects and six placebo subjects. Four of the amlodipine group and three of the placebo group suffered adverse events, resulting in four amlodipine subjects being discontinued from the study (three subjects suffered headaches and one subject an ectopic pregnancy thought not to be treatment related). Compliance determined by tablet counts >80% was satisfactory in all but 15 (nine amlodipine, six placebo) subjects. Scans from three amlodipine and four placebo subjects were withdrawn for technical reasons as already described.

Age, weight and baseline levels of lipid subfractions were comparable in both groups and there were no significant changes in lipid subfractions over the course of the study (Table 1). There was a larger proportion of females in the amlodipine group.

3.2. Vascular function

At baseline, heart rate, resting blood pressure, resting vessel size, brachial artery blood flow, degree of reactive hyperaemia, FMD and dilation to GTN were comparable in the amlodipine and placebo group (Table 1). Amlodipine had no effect on resting heart rate, blood pressure, vessel size, brachial blood flow or the degree of reactive hyperaemia, suggesting that it did not cause significant vasodilatation in this normotensive cohort. There was no significant change in cholesterol levels in either group throughout the study.

In the subjects treated with amlodipine, mean FMD increased from 2.32 ± 2.23% at baseline to 3.24 ± 2.2% and 3.52 ± 3.1% at 12 and 24 weeks, respectively. In the placebo group, FMD also increased from 1.64 ± 2.12 to 2.97 ± 2.98 and 3.37 ± 2.68% (Fig. 1). The increase in FMD within the amlodipine and the placebo groups was significant at both 12 weeks (amlodipine P = 0.006, placebo P = 0.008) and 24 weeks (amlodipine P = 0.005, placebo P = 0.002), but there was no significant difference between responses of the amlodipine and placebo group. Response to GTN did not change significantly in either group over the duration of the study (Fig. 1).

3.3. Determinants of flow and GTN mediated dilatation

On univariate and multiple regression analysis, baseline FMD and GTN-mediated dilatation were significantly related to sex and vessel size but not to age, resting flow, reactive hyperaemia, blood pressure and lipid levels (sex and vessel size are not independent as the mean vessel size of female subjects is 0.69 mm smaller than that of the males). On examining changes in FMD and GTN-mediated dilatation over the study duration, no significant determinants were found even when the treatment group was included as an independent variable, or after correcting FMD for vessel size to account for differences between the groups.

4. Discussion

Despite experimental evidence indicating a potential beneficial effect of the dihydropyridine CCB on the
development of atherosclerosis, in this double-blind study, no favourable effect was found after 6 months treatment with amlodipine, over placebo, on conduit artery physiology, in young clinically well subjects with a strong family history of CAD.

Individuals with a family history of premature CAD are at increased risk of the development of atherosclerosis and its complications, even in the absence of other recognised risk factors [1–6]. The mechanism of increased vascular risk in these subjects is unclear but it is likely that a variety of genes interact to alter the arterial wall and/or its susceptibility to damage from risk factors [11]. Recently, cholesterol lowering has been shown to reduce morbidity and mortality in populations of middle-aged and older subjects even with ‘normal’ cholesterol levels [38]. However, other strategies may have a place in primary and secondary prevention in cohorts with low levels of modifiable risk factors. In our current study, the effect of calcium channel blockade on abnormalities of conduit artery endothelial function was examined in a defined cohort of young preclinical subjects whose only identifiable risk factor was a strong family history of premature coronary artery disease.

There is accumulating evidence for the potential of the CCB as anti-atherogenic agents [28]. Angiographic studies in patients with CAD have demonstrated that CCB can retard the development of atherosclerotic lesions [23,24]. Recent work has also shown a beneficial synergistic effect on atherosclerotic progression, in subjects receiving both CCB and the HMG-CoA reductase inhibitor, Pravastatin [25]. The mechanism by which CCB exert this effect is uncertain but is likely to involve the suppression of SMC proliferation and migration, and connective tissue secretion, all processes dependent on calcium as an intracellular second messenger [39].

We chose the highly lipophilic dihydropyridine CCB, amlodipine, as it has been shown to have potentially important effects in addition to those of other CCB. These include pronounced antioxidant properties [41] and stabilising effects on cell membrane lipid bilayers [27]. This may be the basis of its protective effects on the vascular endothelium, with preservation of endothelium-dependent responses, seen in cholesterol fed primates [31].

Over 6 months, improvement in endothelium-dependent relaxation occurred in both the amlodipine- and placebo-treated subjects. As study subjects were selected on the basis of impaired FMD at baseline, this may have contributed, although we sort to minimise this by not including the screening scan at baseline for statistical comparison. Improvements in both groups might relate to subtle changes in individuals’ behaviour (e.g. increased exercise, change in diet, self-medication with antioxidants) during a clinical trial that is difficult to control or quantify. This might have been particularly prevalent in our population who had been identified with impaired endothelial function at baseline.

The failure to demonstrate a statistically significant difference between amlodipine and placebo may have a number of explanations. Inhibition of calcium as a second messenger is likely to affect smooth muscle cell proliferation and migration, and the secretion of connective tissue components rather than the endothelium. This might have a greater impact later in the atherosclerotic process on advanced fibroproliferative plaques. This may account for the beneficial effects on atherosclerotic plaque progression seen in patients with established disease [23–25]. In this study, subjects were purposely selected with low levels of LDL-cholesterol and no other identifiable risk factors known to increase oxidative stress. This may have limited the benefits
obtainable by reduction of lipid peroxidation and the preservation of superoxide dismutase shown in animal studies with amlodipine [40]. The impact of amlodipine in subjects with cardiovascular risk factors, such as hypercholesterolaemia, or more advanced atherosclerosis remains to be tested. Finally, any demonstration of benefit in these young subjects may require longer-term clinical studies.

Individuals with a strong family history of premature atherosclerosis represent a very heterogeneous population in whom the contribution to vascular risk is poorly understood and is likely to be multifactorial. Further studies of the mechanisms involved in the increased risk of atherosclerosis in these young subjects may lead to the development of specific therapies targeting atherogenesis and its clinical consequences in these young subjects.

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References


