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Oral L-arginine improves endothelium-dependent dilatation and reduces monocyte adhesion to endothelial cells in young men with coronary artery disease

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Abstract

L-Arginine is the physiological substrate for nitric oxide synthesis by the vascular endothelium. In hypercholesterolaemic rabbits, oral L-arginine reduces atheroma, improves endothelium-dependent dilatation and reduces monocyte/endothelial cell adhesion. The effect of oral L-arginine on endothelial physiology is unknown, however, in humans with established atherosclerosis. In a prospective, double-blind, randomised crossover trial, ten men aged 41 ± 2 years with angiographically proven coronary atherosclerosis took L-arginine (7 g three times per day) or placebo for 3 days each, with a washout period of 10 days. After L-arginine, compared to placebo, plasma levels of arginine were increased (318 ± 18 vs. 124 ± 9 μmol/l, $P < 0.01$) and endothelium-dependent dilatation of the brachial artery (measured as the change in diameter in resonse to reactive hyperaemia, using external vascular ultrasound) was improved (4.7 ± 1.1 vs. $1.8 \pm 0.7\%$, $P < 0.04$). No changes were seen in endothelium-independent dilatation of the brachial artery (measured as the change in diameter in response to sublingual nitroglycerine), blood pressure, heart rate or fasting lipid levels. Serum from six of the ten subjects after L-arginine and placebo was then added to confluent monolayers of human umbilical vein endothelial cells for 24 h, before human monocytes obtained by countercurrent centrifiguation elutriation were added and cell adhesion assessed by light microscopy. Adhesion was reduced following L-arginine compared to placebo (42 ± 2 vs. $50 \pm 1\%$, $P < 0.01$). In young men with coronary artery disease, oral L-arginine improves endothelium-dependent dilatation and reduces monocyte/endothelial cell adhesion. © 1997 Elsevier Science Ireland Ltd.

Keywords: Atherosclerosis; Nitric oxide; Leukocytes

1. Introduction

Endothelial dysfunction is an important early event in atherogenesis [1–4] and also determines dynamic plaque behaviour in patients with advanced coronary artery disease [5,6]. In both animal models of atherosclerosis and humans with established arterial disease, there is impairment of endothelium-dependent dilatation and enhanced monocyte adhesion to endothelial cells [6–9]. These abnormalities may be due, at least in part, to a reduction in the availability of endothelium derived relaxing factor [10–12], which has been shown to be nitric oxide [13].

L-Arginine is the physiological substrate for nitric oxide synthesis [13]. In the hypercholesterolaemic rabbit model of atherosclerosis, oral administration of L-arginine reduces atheroma formation, inhibits platelet aggregation, improves endothelium-dependent dilatation and reduces monocyte adhesion to endothelial cells [14–17]. In human studies, we have recently demon-

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strated that oral L-arginine inhibits platelet aggregation in healthy adult men [18] and improves endothelium-dependent dilatation in asymptomatic hypercholesterolaemic subjects [19]. The effects of oral L-arginine on endothelial physiology in subjects with symptomatic and advanced coronary artery disease, however, is not known, nor has the effect of oral L-arginine supplementation on monocyte/endothelial cell adhesion been studied in humans.

Using a prospective, double-blind, crossover trial design, we have therefore assessed the effect of short term oral supplementation with L-arginine on endothelial physiology and endothelial/monocyte adhesion in a group of young men with advanced coronary atherosclerosis.

2. Methods

2.1. Study design

Ten men aged 41 ± 2 years were studied. Subjects were included if they were aged less than 55 years, if they had angiographically documented coronary artery disease in at least two vessels, had not actively smoked for at least 2 years, were non-diabetic and were taking no vasoactive medications. Subjects on other medications were included provided they had been on a stable dose for at least 6 months. Subjects were asked not to alter their medical treatment for the duration of the study. Subjects were only included if their endothelium-dependent dilatation was less than 1 standard deviation below for the mean value documented in healthy controls [20] on baseline assessment. The study was approved by the institutional ethics committee, and each subject gave written informed consent.

Each subject was seen on three occasions; at baseline, and after 3 days of either oral L-arginine or placebo. At each visit, blood was collected onto ice, for biochemical studies (total cholesterol, triglycerides and high density lipoprotein cholesterol, liver function tests, arginine and citrulline levels) and nitrosoprotein levels. Serum for cell culture experiments was collected in procoagulant-containing tubes (SST gel and clot activator vacutainer, Becton Dickinson, Rutherford, NJ), immediately centrifuged (3 000 revs./min for 10 min) at 4°C and stored at -80°C until use. Heart rate, blood pressure and vascular reactivity of the brachial artery were then measured, as below.

Following baseline tests, the subjects were randomised to receive either placebo or L-arginine powder (Scientific Hospital Supplies, Liverpool, UK). L-arginine was administered as a pineapple flavoured powder dissolved in water at a dose of 7 g, three times a day, taken with meals, for 3 days. This treatment regime was chosen, as we have previously shown that 3 days of L-arginine reduces platelet aggregation but does not enhance endothelial function in healthy young men [18]. Placebo powder was matched for flavour and appearance, and did not contain any protein. There was a 10–14 day washout period between the alternate medications. Blood and haemodynamic tests were performed 1–1.5 h after the last dose of each powder in each case.

2.2. Biochemical studies

Total cholesterol and triglycerides were measured enzymatically using a Hitachi 747 autoanalyzer, high-density lipoprotein levels were determined after dextran sulfate magnesium precipitation and low-density lipoprotein cholesterol was calculated using the formula described by Friedwald et al., [21]. Arginine and citrulline levels were measured after deproteinising media with 2% sulphur salicylic acid using high performance liquid chromatography.

2.3. Nitrosoprotein assay

Nitric oxide normally circulates in the plasma partially complexed to thiol groups of proteins [22], and nitrosoprotein levels in plasma are therefore thought to reflect endothelial nitric oxide production. It should be noted, however, that other nitric oxide products are present in the circulation, such as nitrate [23], and thus no single assay can give a quantitative estimate of circulating nitric oxide; we measured circulating nitrosoproteins as an indicator of the endogenous plasma levels of bioavailable nitric oxide [22]. In this study, nitrosoproteins were determined in freshly prepared plasma after displacement of nitric oxide with Hg^{2+}, by diazotisation of sulphanilamide followed by coupling with *N*-(1-naphthyl)-ethylene diamine, according to the method of Saville [24]. Blood samples were collected into heparin on ice, immediately transported to the laboratory and centrifuged for 20 min at 4°C to remove cells. The supernatant plasma was removed to a separate tube on ice and assayed immediately for protein-bound nitric oxide.

2.4. Brachial artery vascular reactivity

Endothelial function was assessed non-invasively by measuring endothelium-dependent dilatation, as described previously [3]. High resolution external ultrasound ($128 \times P/10$ with 7.0 MHz linear array transducer, Acuson, Mountain View, CA) was used to measure changes in brachial artery diameter in response to reactive hyperaemia and in response to glyceryltrinitrate. The right brachial artery was scanned in longitudinal section 2–15 cm above the elbow (control scan), after at least 10 min rest in the supine position. Depth

and gain settings were optimised to identify the vessel wall to lumen interface, and a resting scan recorded. Arterial flow velocity was measured at rest using pulsed-wave Doppler, and flow calculated from the velocity-time integral, the heart rate and the vessel diameter. Hyperaemia was induced by inflation and then deflation of a pneumatic cuff placed around the forearm (below the scanned part of the artery), inflated to approximately 250 mmHg for 4.5 min. The artery was scanned prior to cuff inflation and for 90 s after cuff deflation. Flow-mediated dilatation measured in this way has been shown to depend on endothelial production of nitric oxide [25,26] and to correlate closely with endothelial function in the coronary circulation [27]. Flow velocity was remeasured during the first 15 s after cuff deflation, and reactive hyperaemia was calculated as the ratio of the post-deflation flow to the resting flow value. After 10 min rest a further control scan was recorded and finally 400 μg of glyceryltrinitrate spray was administered. The final scan was performed 3 min after glyceryltrinitrate. All scans were recorded on super-VHS tape and later analysed by two independent observers blinded to the identity of the subject, the scan sequence and the stage of the experiment. For calculation of flow-mediated dilatation, the vessel diameter at 50–60 s after cuff deflation was divided by the average control diameter. For each subject, the average value of the two observers for both flow-mediated and glyceryltrinitrate-induced dilatation is presented. It has previously been shown that this technique of measurement and analysis is both accurate and reproducible under the conditions described here [28].

2.5. Endothelial cell culture

Human umbilical vein endothelial cells were harvested enzymatically, using a type II collagenase under sterile conditions, as described by Minter et al. [29], and established as primary cell cultures in M199 (Trace Biosciences, Australia) containing 20% heat inactivated human serum, L-glutamine 2 mM (ICN Biomedicals, Lisle, IL), 0.5% endothelial cell growth promoter (Starrate Products, Bethungra, NSW), penicillin 100 U/ml and streptomycin 0.1 mg/ml. All media were prepared using endotoxin-free water (Baxter, Lane Cove, Australia) and filtered using Zetapore filters (Cuno Life Sciences Division, Meriden, CT). Endotoxin-free plasticware and glassware were used in all experiments.

For experimental studies, confluent human umbilical vein endothelial cell monolayers (passage 1–4) were trypsinised and replated on to gelatin-coated 24-mm diameter tissue culture plates for monocyte adhesion studies (Falcon, Becton Dickinson, Lincoln Park, NJ). Plates were gelatin coated by adding 1 ml per 5 cm^2 of Haemaccel (Behringwerke, Marburg, Germany) diluted 1:250 in phosphate buffered saline, incubating for 1 h at 37°C, then decanting solution prior to use. Human umbilical vein endothelial cells were grown to confluence and used within 72 h. Prior to use, each monolayer was inspected microscopically to ensure that only endothelial cells were present and purity of the cultures was periodically confirmed by staining cells using a monoclonal antibody specific for Von Willebrand factor.

2.6. Isolation of human monocytes

White cell concentrate (Red Cross Blood Bank, Sydney, Australia) was obtained from peripheral blood of human volunteers, who had no clinical evidence of cardiovascular disease. Monocytes were isolated within 24 h of collection, by density gradient separation of white cell concentrate anticoagulated with 0.07% EDTA (Merck Pty, Kilsyth, Australia) on Lymphoprep (Nycomed Pharma, Oslo, Norway) at 20°C followed by counterflow centrifugation elutriation, as described by Garner et al. [30]. A Beckman J2-21M/E centrifuge was used equipped with a JE-6B elutriation rotor and a standard 4.2 ml elutriation chamber (Beckman instruments, Palo Alto, CA). The elutriation buffer was Hank's balanced salt solution (HBSS) without calcium or magnesium (Trace Biosciences, Australia), with EDTA (0.1 g/l) and 1% heat inactivated human serum added. Following rinsing of the system with 250 ml of 70% ethanol, 250 ml endotoxin-free water, 250 ml of 6% hydrogen peroxide, another 250 ml endotoxin-free water and 250 ml of elutriation buffer, the mononuclear cell fraction taken from the Lymphoprep density gradient at the Lymphoprep-plasma interface was loaded at 9 ml/min into the elutriation rotor (2020 revs./min at 20°C). Flow rate was increased by 1 ml/min every 10 min and fractions of eluted cells collected. Monocytes were eluted between 16 and 17 ml/min. Collected cell fractions were examined using a Cytospin system (Shandon, Pittsburgh, PA) and Wright's stain (Diff-Quik, LAB-AIDS). Monocyte suspensions were only used if purity was $>90\%$ on light microscopy, with less than 1% contamination by neutrophils, and viability $>95\%$ by Trypan blue exclusion. Monocytes were then resuspended in RPMI containing 10% heat inactivated human serum, penicillin (100 U/ml) and streptomycin (0.1 mg/ml), stored in teflon containers at a concentration between $1.5–2.0 \times 10^6$/ml at 37°C under 5% CO_2 in air and used within 3 h.

2.7. Monocyte-endothelial adhesion studies

Confluent endothelial monolayers were incubated with arginine-free RPMI medium (Life technology, Gaithersburg, MA) containing 50% serum from six randomly selected subjects out of the ten participants.

Each of these subjects had serum collected after both placebo and L-arginine therapy. In each case, the medium was added to the endothelial cell monolayers for 24 h prior to adhesion assays. At the end of the 24 h incubation, cell viability was greater than 95% (by Trypan blue exclusion) for each subject for each condition. Experiments were repeated on at least two occasions, with four separate wells used for each condition on each subject. Following initial experiments the adhesion assays were repeated with 100 μM L-NMMA added to the wells containing serum taken from subjects after L-arginine supplementation.

The adhesion assay was performed by adding 1 ml of monocyte suspension (in RPMI with 50% human serum from the condition being tested) at a concentration of $1.0–1.5 \times 10^6$/ml to each endothelial cell monolayer and incubating for 1 h at 37°C under 5% CO_2 in air. After 1 h, non-adherent cells were removed by standardised gentle washing using a 1000-μl automatic pipette (Gilson) and the suspension stored on ice until the cell concentration was counted using a Neubauer haemocytometer (Weber Scientific, Middlesex, UK). The initial suspensions and the suspensions from each well were counted four times. The percentage of adherent monocytes was then calculated by comparison with the initial monocyte concentration.

Prior to this study, preliminary experiments were conducted to assess the effect of varying time post elutriation on monocyte adhesion to cultured endothelial cells. These studies were carried out three times on 3 separate weeks using 12 wells for each condition. Intra-observer error was also assessed in these initial studies, with repeated measurements taken on separate wells under the same conditions, and repeated measurements taken on the same wells, separated by time. Basal monocyte adhesion to HUVEC was not significantly different at 1 h (38%), 24 h (35%), or 48 h (35%) post elutriation. This technique had a low intraobserver error, with a coefficient of variation of $< 5\%$.

2.8. Statistics

Data were analysed with SPSS for Windows 6.0 (Chicago, IL). All descriptive data are expressed as mean $\pm$ standard error of the mean. An 'order effect' related to treatment randomisation was excluded by comparing the results from the six subjects receiving L-arginine first to those from the four subjects receiving placebo first. Using independent samples t-tests, there were no significant differences in the baseline or the post-supplementation data between these two groups. Thereafter, data were assessed using repeated measures analysis of variance (ANOVA), followed by Scheffe's procedure for multiple comparisons. Accordingly P-values in the results section refer to the comparison of the results following L-arginine to those following placebo. The prospectively defined primary endpoints were change in flow-mediated dilatation and change in monocyte/endothelial cell adhesion. Statistical significance was inferred at a two-sided P-value of < 0.05.

3. Results

The results are summarised in Table 1. The oral L-arginine was tolerated well by all subjects with no significant side-effects. All subjects included in the trial completed the trial protocol. Self- reported compliance was $> 95\%$ overall and 100% for the final doses of either L-arginine or placebo.

3.1. Subjects

Fifteen subjects with angiographically proven coronary artery disease were initially assessed at baseline, with only ten fulfilling the inclusion criteria (see Section 2). Of the ten subjects included, nine were ex-smokers and one had never smoked; the mean cigarette smoke exposure was 13 ± 2.9 pack years. Five subjects had a family history of premature vascular disease (coronary artery disease in a first degree relative less than 55 years of age) and five had previously been diagnosed as having hypercholesterolaemia (all five of these subjects were recieving treatment with a HMG CoA reductase inhibitor and all had been on a stable dose for at least 1 year). None of the subjects had a history of hypertension or diabetes mellitus. All subjects had previously had coronary artery disease documented by coronary angiography, with at least two vessels having a $> 70\%$ stenosis. Eight of these subjects had had coronary artery bypass grafts and one had had percutaneous transluminal angioplasty performed. The minimum du-

Table 1
Biochemical and haemodynamic studies at baseline and following 3-days supplementation with placebo or L-arginine

	L-Arginine	Placebo
L-Arginine (μmol/l)	318 ± 18*	124 ± 9
Urea (mmol/l)	6.7 ± 1.3**	5.9 ± 0.9
Total cholesterol (mmol/l)	5.9 ± 1.2	5.9 ± 0.9
LDL cholesterol (mmol/l)	3.5 ± 2.7	3.4 ± 2.1
HDL cholesterol (mmol/l)	0.76 ± 0.18	0.79 ± 0.2
Triglycerides (mmol/l)	3.3 ± 2.2	3.1 ± 2.1
NO proteins (μmol/l)	3.0 ± 3.2	0.4 ± 1.1
Blood pressure (mmHg)	$131/78 \pm 15/8$	$116/68 \pm 43/26$
Heart rate (beats/min)	72 ± 10	69 ± 9
Vessel size (mm)	3.7 ± 0.6	3.7 ± 0.7
Flow-mediated dilatation (%)	4.7 ± 1.1**	1.8 ± 0.7
Glyceryltrinitrate response (%)	14.7 ± 5.4	13.5 ± 5.0

LDL, low density lipoprotein; HDL, high density lipoprotein; NO proteins, nitrosothiol estimation.
*$P < 0.001$, **$P < 0.04$ by repeated measures ANOVA (see Section 2).

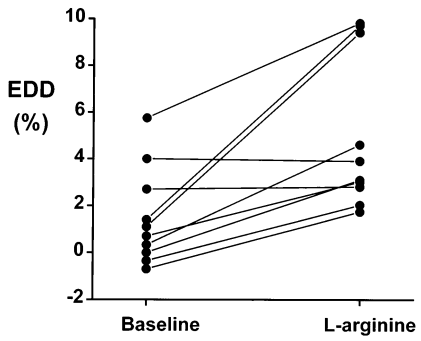

Fig. 1. Endothelium-dependent dilatation (EDD) at baseline and after 3 days of oral L-arginine supplementation ($P < 0.04$).

ration post procedure was 18 months. All were clinically stable and well, and none had clinical evidence of cardiac failure. Nine of the subjects were taking aspirin daily and one subject was taking warfarin. None were taking anti-anginal or anti-failure medications, and none had a history of any other systemic medical illnesses.

3.2. Biochemical studies

There was a significant rise in plasma levels of L-arginine after supplementation with L-arginine but not after placebo (318 ± 18 μmol/l after L-arginine compared to 124 ± 9 μmol/l after placebo, $P < 0.001$). Urea levels also rose with L-arginine (6.7 ± 0.4 after L-arginine compared to 5.0 ± 0.3 after placebo, $P < 0.003$). There was no difference in citrulline levels after L-arginine compared to placebo. At baseline the mean total cholesterol was 5.9 ± 0.9 mmol/l, LDL cholesterol 3.4 ± 2.6 mmol/l, HDL cholesterol 0.83 ± 0.23 mmol/l and triglycerides 3.5 ± 2.5 mmol/l. None of the measured lipid parameters changed significantly after L-arginine compared to placebo.

3.3. Nitrosoprotein assay

Nitrosoprotein levels tended to be higher following L-arginine compared to placebo, however this was not statistically significant (3.5 ± 1.34 μmol/l after L-arginine compared to 0.5 ± 0.5 μmol/l after placebo, $P = 0.09$).

3.4. Vascular reactivity studies

There were no significant changes in baseline vessel size, degree of hyperaemia, resting blood flow, blood pressure or heart rate after L-arginine compared to placebo. Following L-arginine there was a significant improvement in endothelium-dependent dilatation from baseline (Fig. 1); after placebo however, there was no significant change (4.7 ± 1.1 vs. $1.8 \pm 0.7\%$, $P < 0.04$). By contrast, no change was seen in the degree of dilatation to glyceryltrinitrate (Table 1). There were no significant relationships between the degree of endothelium-dependent dilatation observed after L-arginine and the plasma levels of L-arginine, serum cholesterol or medications being taken.

3.5. Monocyte/endothelial adhesion:

Monocyte adhesion to endothelium was lower when endothelial cell monolayers were pretreated with serum taken after L-arginine supplementation compared to placebo (42 ± 2 vs. $50 \pm 1\%$, $P < 0.01$) (Fig. 2). This effect was reversed by the addition of 100 μM L-NMMA to the post L-arginine serum, during the 24-h incubation period ($49 \pm 3\%$ vs. $42 \pm 2\%$ without L-NMMA).

4. Discussion

Endothelial production of nitric oxide and monocyte adhesion to endothelial cells are both importantly involved in the atherogenic process. In this study, we have shown that it is possible to improve nitric oxide-dependent endothelial function with oral L-arginine therapy, even in young men with advanced coronary artery disease. Furthermore, ex vivo studies of monocyte/endothelial cell adhesion suggest a reduction in adhesion after L-arginine. This reduction in monocyte/endothelial cell adhesion could be blocked by the addition of *N*-monomethyl-L-arginine, a specific nitric oxide synthase inhibitor, consistent with a direct L-arginine-dependent effect, possibly mediated via the L-arginine/nitric oxide pathway.

4.1. Endothelial effects

The effect of oral L-arginine on endothelial physiology in humans with established coronary artery disease has not been reported previously. In cholesterol-fed rabbits, it has been shown that dietary L-arginine preserves endothelium-dependent dilatation induced by acetylcholine when L-arginine is administered from the start of cholesterol feeding [14,15]. In this hypercholesterolaemic rabbit model, L-arginine supplementation also decreased atheroma formation and reduced platelet aggregation [14,16]. Previous studies of L-arginine in asymptomatic human subjects have similarly demonstrated beneficial effects. In humans, acute intravenous administration of L-arginine improves endothelial physiology in hypercholesterolaemic subjects [31]. Furthermore, we have recently reported that 4 weeks supplementation with oral L-arginine significantly improves endothelium-dependent dilatation in hypercholesterolaemic young adults with no known vascular

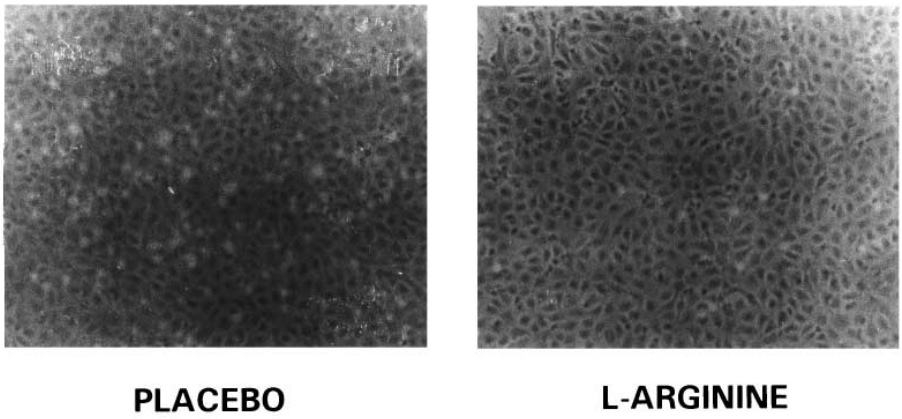

Fig. 2. Photomicrographs showing monocyte adhesion to HUVEC monolayers, taken randomly from the centre of 25-mm wells. The monolayer on the left has been incubated for 24 h in 50% serum taken at baseline. The monolayer on the right has been incubated for 24 h in 50% serum taken from the same subject after 3 days of oral L-arginine supplementation. Overall adhesion was significantly lower after L-arginine compared to placebo (42 ± 2 vs. $50 \pm 1\%$, $P < 0.01$).

disease [19] and that short term oral L-arginine inhibits platelet aggregation in healthy subjects [18]. Much less work has addressed the effects of L-arginine in subjects with advanced atherosclerosis. Otsuji et al. recently observed that an infusion of L-arginine prevented acetylcholine-induced vasoconstriction if coronary vessels were smooth on angiography but not if vessels were irregular [32]. In contrast, Drexler et al. found that intracoronary L-arginine could improve endothelium-dependent dilatation in the coronary microcirculation of patients with symptomatic coronary artery disease [33]. Our study, however, demonstrates that oral L-arginine may improve endothelium-dependent dilatation in the large systemic arteries, even in subjects with severe coronary artery disease.

4.2. Cell adhesion effects

Monocyte adhesion to endothelial cells was also altered by oral L-arginine therapy, in this study. Many investigators have demonstrated that levels of monocyte/endothelial cell adhesion can be increased in vitro by exposure to various factors, such as β-VLDL, minimally modified LDL and extracts of cigarette smoke [34–36]. Some in vitro studies have shown that these changes in monocyte/endothelial cell adhesion can be modified by concurrent incubation with nitric oxide donors [37] or antioxidant vitamins [38]. In cholesterol-fed rabbits, Tsao et al. have reported that monocyte binding to endothelial cells is reduced in those animals treated with L-arginine [17]. In the current human study, monocyte/endothelial cell adhesion was reduced when HUVEC monolayers were pretreated with serum from subjects supplemented with L-arginine compared to placebo. It is possible that the increased concentration of L-arginine led to an improvement in nitric oxide production by the HUVEC or that L-arginine supplementation resulted in other changes in the serum. It is unlikely that this effect is due to increased nitric oxide within the serum, due to its extremely short half-life [25].

4.3. Mechanism of action

The mechanism of the beneficial actions of L-arginine, the physiological substrate for nitric oxide synthesis, is largely unknown. In atherosclerosis, as well as in the presence of risk factors for atherosclerosis, there appears to be a relative decrease in the availability of nitric oxide in the arterial wall [10–12]. There is also some evidence from animal studies that nitric oxide production decreases with aging and that this may be accompanied by a reduction in plasma L-arginine levels [39].

Several authors have suggested that increasing L-arginine levels to supraphysiologic levels may improve the availability of endothelium-derived nitric oxide. Although in vitro studies of the dissociation constant of L-arginine for nitric oxide synthase suggest that production of nitric oxide should not be substrate-limited [40] and L-arginine supplementation does not seem to increase nitric oxide production in normal subjects [18,31,41], the situation may differ in the in vivo situation and in the presence of vascular damage. For example, the affinity of nitric oxide synthase for L-arginine varies in the presence of other amino acids [42] and it has been shown that other factors, such as coincubation with oxidised LDL, may influence cellular transport of L-arginine as well as the expression of nitric oxide synthase [43].

L-Arginine may have other effects to explain its beneficial actions in vivo, such as inhibition of lipid peroxidation [44,45]. De Caterina et al. have recently demonstrated that nitric oxide donors are capable of

modulating the surface expression of the cell adhesion molecules VCAM-1, ICAM-1 and E-selectin [46], and that this is probably mediated through the inhibition of NF-κB. In vitro studies in our laboratory have examined L-arginine's effects on monocyte/endothelial cell adhesion, and suggest that L-arginine may also alter endothelial cell expression of ICAM-1 and VCAM-1 at a transcriptional level [47]. The observed changes in monocyte/endothelial cell adhesion in the present ex vivo study may similarly be due to alterations in the expression of cell adhesion molecules.

4.4. Limitations

In this study, we investigated endothelial physiology in only younger subjects with coronary artery disease, as we have previously shown that endothelium-dependent dilatation declines after age 55 years, even in healthy subjects with no vascular risk factors (that is, only younger subjects with coronary artery disease might show significant reversibility towards 'normal', using our method for testing arterial reactivity) [48]. The improvements in endothelial function seen in the current study, therefore, are only applicable to younger subjects with coronary artery disease who have abnormal flow-mediated dilatation at baseline. We have previously demonstrated that 3 days of L-arginine supplementation has no significant effect on arterial reactivity in healthy subjects with normal endothelium-dependent dilatation [18]. In this latter study we also investigated the effects of oral L-arginine on platelet function; this could not be done in the present study, as nine of the ten patients were prescribed regular antiplatelet therapy. In this study we did, however, investigate monocyte/endothelial cell interactions, using highly purified populations of human cells and human serum. This model may not accurately reflect the in vivo situation in the vascular wall. For example, HUVEC do not usually express ICAM-1 or VCAM-1 in the basal state [46], whereas cell adhesion molecule expression is usually present on endothelial cells found in atherosclerotic plaque [49]. Despite this, HUVEC have been used by many investigators, and seem to have similar in vitro characteristics to arterial endothelial cells [29]. The model used in our study primarily assesses the effect of L-arginine on endothelial adhesiveness and does not examine possible differences in the monocytes of these subjects.

The dosage of L-arginine used in this and our previous studies is similar (dose/kg) to that used in reported animal studies [14–17], and is the highest dosage well tolerated in humans [18,19]. It is possible that a different regime may have different effects to that observed in our study. Although we observed a significant improvement in endothelium-dependent dilatation with L-arginine supplementation (from $\sim$2 to $\sim$5%), it was not restored to normal range of values expected in age matched controls (7–9%) [20]. This may have been due to other potentially reversible factors which are not altered by L-arginine, or because the vascular damage may not be completely reversible in subjects with advanced atherosclerosis. In these subjects, we assessed endothelial function non-invasively in a peripheral conduit artery, rather than their coronary endothelial physiology. It has recently been shown, however, that endothelial function in the brachial artery is closely correlated with that in the coronary circulation [27]. Furthermore, ultrasound-based measurement of flow-mediated, endothelium-dependent dilatation of the brachial is known to be accurate, reproducible and is mainly due to endothelial release of nitric oxide [25,26].

5. Conclusion

In young adult men with advanced coronary artery disease, oral L-arginine may improve endothelial dysfunction and reduce monocyte/endothelial cell adhesion. As in animal models of atherosclerosis, this may impact favourably on the atherogenic process. Whether the observed benefits of such therapy in humans are sustained with prolonged therapy requires further prospective evaluation.

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