High-Dose Allopurinol Improves Endothelial Function by Profoundly Reducing Vascular Oxidative Stress and Not by Lowering Uric Acid

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Background—Allopurinol has been shown to improve endothelial function in chronic heart failure. This study aimed to establish its mechanism of action and to construct a dose–response curve for the effect of allopurinol.

Methods and Results—Two randomized, placebo-controlled, double-blind, crossover studies were performed for 1 month on patients with New York Heart Association Class II–III chronic heart failure, comparing 300 mg allopurinol, 600 mg allopurinol, and placebo for the first study and 1000 mg probenecid versus placebo in the second study. Endothelial function was assessed by standard forearm venous occlusion plethysmography. Allopurinol 600 mg/d significantly increased forearm blood flow response to acetylcholine compared with both allopurinol 300 mg/d and placebo (% change in forearm blood flow [mean±SEM]: 240.31±38.19% versus 152.10±18.21% versus 73.96±10.29%, \( P<0.001 \)). For similar levels of urate lowering, the uricosuric agent probenecid had no effect on endothelial function. Sodium nitroprusside response was unchanged by all treatments. Vitamin C and acetylcholine coinfusion data showed that 600 mg/d allopurinol completely abolished the oxidative stress that was sensitive to high-dose vitamin C.

Conclusions—For the first time, we have shown that a steep dose–response relationship exists between allopurinol and its effect on endothelial function. We also showed that the mechanism of improvement in endothelial function with allopurinol lies in its ability to reduce vascular oxidative stress and not in urate reduction. The reduction in vascular oxidative stress was profound because high-dose allopurinol totally abolished the oxidative stress that was sensitive to the high-dose vitamin C that was used in this study. (Circulation. 2006;114:2508-2516.)

Key Words: endothelium ■ free radicals ■ heart failure ■ allopurinol ■ uric acid

Numerous clinical studies have shown that xanthine oxidase (XO) inhibition improves endothelial function in patients with diabetes, patients with coronary artery disease, smokers, and, in particular, patients with chronic heart failure (CHF).1–4 Allopurinol is a potent XO inhibitor and is commonly used worldwide for the treatment of gout. However, the pharmacodynamics of allopurinol are complex because XO forms 2 very different molecules, uric acid and free radicals. Reductions in the latter should in theory reduce oxidative stress and improve endothelial function. By contrast, it is difficult to predict what effect reductions in urate might have. This is because high urate levels are independently associated with a worse prognosis in a wide cohort of patients with cardiovascular disease.5–7 On the other hand, the urate molecule has antioxidant activity in vitro, but it is unknown if this activity is relevant in vivo.8–10 Urate levels could therefore be merely a marker of XO activity and the harm to endothelial function is actually caused by XO-induced oxidative stress. Indeed, if the urate molecule really does have antioxidant activity, the fall in urate produced by XO inhibition could in theory detract from the ability of allopurinol to improve endothelial function.

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The present study set out to establish the mechanism by which allopurinol improves endothelial function in CHF. We also set out to address another key question about the possible therapeutic use of allopurinol, ie, what is the dose–response curve between allopurinol and its effect on endothelial function?

Dose–response curves are an essential component in the development of any drug in order to ensure that its optimum dose is used in subsequent end point studies. Dose–response curves are particularly pertinent for XO inhibitors for several reasons. First, a previous retrospective observational study of allopurinol suggests that 300 mg allopurinol may reduce cardiovascular events in CHF patients to a greater extent than 100 mg allopurinol.11 Second, in treatment of gout, allopuri-
Allopurinol can be given up to 900 mg/d according to the British National Formulary; yet in routine clinical practice and in virtually all clinical research, 300 mg/d is the usual daily maximum. Third, studies using oxypurinol have produced conflicting results showing either a positive effect on left ventricular (LV) remodeling (unpublished data from the La Plata study and the Evaluation of XanThine Oxidase Inhibition on Cardiac Ejection Fraction [EXOTIC-EF] study; Cardiomed Inc. press release) and neutral results (Oxypurinol Therapy for Congestive Heart Failure [OPT-CHF]) on hospitalizations for patients with advanced heart failure. These conflicting results suggest that a borderline effective dose of oxypurinol may have been used in these studies.

Methods

Two studies were carried out in the Department of Clinical Pharmacology at Ninewells Hospital in Dundee, UK. Patients were recruited from Specialist Heart Failure Clinics and General Cardiology Outpatient Clinics in Dundee and surrounding towns. All subjects gave written informed consent to participate in the study, which had prior approval by the Tayside Committee on Medical Research Ethics and was conducted in accordance with the Declaration of Helsinki.

All patients underwent screening by clinical history, physical examination, routine hematology and biochemistry analysis, and screening echocardiogram and ECG. Subjects were excluded if they had serum creatinine >2.03 mg/dL, recent admission to hospital within the last 3 months, uncontrolled hypertension (blood pressure >160/90 mm Hg), were on warfarin (procedural risk), were already taking vitamin supplementation, or were on allopurinol or had a previously documented adverse reaction to allopurinol.

Study 1: Dose–Response Study of Allopurinol

Study Population

Thirty patients with stable mild to moderate CHF (New York Heart Association [NYHA] class II–III) with documented LV systolic dysfunction on echocardiogram were studied.

Study Protocol

After initial screening, 30 subjects were given 300 mg allopurinol (morning) and placebo (night), 300 mg allopurinol BID (total 600 mg/d), or placebo twice daily in a randomized, double-blind, crossover fashion. After each 4-week treatment period, each subject attended a 4-hour study morning for assessment of their endothelial function. Mornings were selected to avoid the recognized diurnal fluctuations in endothelial cell function. Patients fasted overnight and were not allowed to have caffeinated drinks or smoke for 12 hours before the tests. Patients took all their usual medications in the same way on each study day except for diuretics (this was done for practical reasons to minimize the need for micturition during the endothelial function testing period). All other medications remained stable during the study period.

Patients attended a temperature-controlled laboratory (24 to 26°C) in our research unit. After 20 to 30 minutes of supine rest, venepuncture for renal function, liver function, C-reactive protein [CRP], lipids, glucose, insulin, uric acid, brain natriuretic peptide (BNP), and procollagen type III N-terminal peptide (PIIINP) was performed. Subjects then underwent cannulation of the nondominant brachial artery with a 27-gauge steel needle (Coopers Needleworks Ltd, Birmingham, Warks, UK) mounted onto a 16-gauge epidural catheter (Portex Ltd, Kent, UK) under local anesthesia. All procedures and data analysis were performed by a single researcher to eliminate interobserver variability. After a period of 30 minutes, during which physiological saline was infused (Graseby 3100 syringe pump, Watford, Herts, UK) at a constant rate of 1 mL/min to allow resting blood flow to stabilize, baseline forearm blood flow (FFB) was measured by means of forearm venous occlusion plethysmography.

This technique has been extensively described in the literature.1,2,14–19

When resting flows were established, FFB was measured during the last 2 minutes of 7-minute drug infusions. Drugs infused were acetylcholine 50 and 100 nmol/min (Novartis, Basel, Switzerland), sodium nitroprusside 12.6 and 37.8 nmol/min (Mayne Pharma, Leamington, Warks, UK), and finally infusion of vitamin C 25 mg/mL with acetylcholine 50 and 100 nmol/min for 7 minutes each at a constant rate of 1 mL/min (Graseby 3100 syringe pump). Each drug infusion period was separated by a washout period (10 to 30 minutes) with 0.9% saline to allow flows to normalize.

All FBF values were expressed as milliliters/minute per 100 mL forearm tissue volume. This was converted to the ratio of the increase in blood flow between the infused arm and the blood flow in the control arm and expressed as a percentage change in FBF (mean ± SEM), calculated according the method described by Whitney.20 The advantage of using noninfused arm data is that confounding effects on blood flow changes by external factors can be minimized.19 The disadvantage of this method is a possibility that the measurement error may be greater because the readings are taken from 2 separate arms. Blood pressure was measured by a semiautomated, noninvasive oscillometric sphygmomanometer (Critikon Dinamap 1846 SX; Critikon, Tampa, Fla) in the noninfused arm before each infusion period and at the conclusion of the study. Plasma levels of insulin (DiaSorin, Wokingham, Berks, UK), PIIINP (Oxford Biosystems Ltd, Oxford, UK), and BNP (Bachem Ltd, St Helens, Merseyside, UK) were all measured at our laboratory using radioimmunoassay.

Statistical Analysis

FFB measurements for individual subjects were compared by means of repeated-measures ANOVA and the Bonferroni method for calculating 95% CI, correcting for multiple comparisons for within-group effects, treatment order effects, and carryover effects. All other outcome data were analyzed by ANOVA. A probability value of P<0.05 was considered significant and a value of P<0.01 was considered highly significant. All results of clinical parameters were expressed as mean±SD and data from forearm venous occlusion plethysmography were expressed as mean±SEM. The data were analyzed by using SPSS for Windows v11.0 (SPSS Inc, Chicago, Ill).

Study 2: Urate Lowering With Probenecid Study

Study Population

An initial pilot study of 10 patients with stable mild to moderate CHF indicated that 1000 mg probenecid would produce urate lowering similar to allopurinol. Twenty-six patients with stable mild to moderate CHF (NYHA class II–III) with documented LV systolic dysfunction on echocardiogram were studied using probenecid 1000 mg versus placebo.

Study Protocol

After initial screening, 26 subjects were given 500 mg probenecid (Benemid, AstraZeneca, Mölndal, Sweden) (morning) and placebo (night) for 1 week followed by a second treatment pack of 500 mg probenecid BID for 3 weeks in the treatment phase. The placebo tablets matched the dosing frequency of the probenecid phase (placebo twice daily for 1 week followed by a second treatment pack of placebo twice daily) in a randomized, double-blind, crossover fashion. This was done to satisfy pharmaceutical advice to gradually increase probenecid dose in order to minimize side effects, as the main aim of this study was to achieve a comparable degree of urate lowering. This worked successfully without any difficulties. The endothelial function study protocol and statistical analysis were similar to study 1 described above. The paired Student t test was used to compare all other outcome data between the 2 groups.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
Results

Study 1: Dose–Response Study of Allopurinol

In general, all doses of allopurinol were well tolerated by the patients. One patient developed a rash with 300 mg allopurinol and withdrew from the trial before any vascular assessment was performed. The baseline clinical characteristics were summarized in Table 1. All subjects were on optimum tolerated medical therapy. None of the patients had taken any vitamin supplementation. All patients were in sinus rhythm at the time of the study, and all had evidence of mild to moderate LV systolic dysfunction documented by echocardiography.

The underlying cause of CHF in 29 subjects was ischemic heart disease. One subject had CHF secondary to previous alcoholic cardiomyopathy.

Biochemical Parameters

Allopurinol lowered plasma uric acid significantly (placebo 0.41±0.11 mmol/L; 300 mg allopurinol 0.23±0.05; 600 mg allopurinol 0.16±0.06; P<0.001). There were no significant differences in serum urea, creatinine, plasma PIIINP, total cholesterol, HDL, CRP, insulin, and glucose among the 3 dosing regimens. For BNP analysis, we subdivided the patients according to NYHA class. There was no difference in BNP levels among all 3 dosing regimens for patients within each NYHA class and between NYHA classes.

Basal Flows, Washouts, and Blood Pressure

Baseline FBF levels in the placebo, 300 mg allopurinol, and 600 mg allopurinol groups were not significantly different (2.02±0.11 versus 2.01±0.10 versus 2.04±0.10 mL/min per 100 mL; P=0.79). There was also no significant difference between the washout periods between the infusions for all 3 treatment periods. For each treatment phase, there was also no significant difference between all the washout periods between the infusions for all 3 treatment periods. For each treatment phase, there was also no significant difference between the baseline and washout FBFs. Systemic blood pressures did not differ between baseline and washout periods.

Vascular Responses to Acetylcholine, Sodium Nitroprusside, and Vitamin C Coinfusion With Acetylcholine

There was a highly significant improvement in endothelium-dependent vasodilation in both allopurinol treatment groups as compared with placebo alone (P<0.001). There was also a highly significant improvement seen with 600 mg allopurinol compared with 300 mg allopurinol (P<0.001) (maximal absolute value [% change in ratio from baseline] 600 mg allopurinol 11.19±1.17 [240.31±38.19] versus 300 mg allopurinol 7.33±0.65 [152.10±18.21] versus placebo 3.88±0.36 [73.96±10.29] mL/min per 100 mL forearm tissue) (Figures 1 and 2).

The mean flow response to acetylcholine (both doses combined) for placebo, 300 mg allopurinol, and 600 mg allopurinol was 3.01±0.41, 4.81±0.41, and 7.33±0.42 mL/min per 100 mL forearm tissue, respectively. This equates to a 59% improvement in FBF between placebo and 300 mg allopurinol and a further 52% improvement in FBF between 300 mg allopurinol and 600 mg allopurinol (143% improvement between placebo and 600 mg allopurinol). This improvement was maintained when the data from the noninfused arm were used (P<0.001). The data between placebo and 300 mg are consistent with our previously published data.
showing approximately 51% improvements in maximal ratio values.1

This benefit was maintained when the cohort was divided according to baseline urate (0.42 mmol/L). In the low uric acid group, there was a significant improvement in absolute FBF between 300 and 600 mg allopurinol (P=0.02) and between placebo and 600 mg allopurinol (P<0.001). In the high uric acid group, the results were similar, with a significant improvement in absolute FBF between 300 and 600 mg allopurinol (P=0.05) and between placebo and 600 mg allopurinol (P<0.001).

There were no significant differences in the FBF responses to the endothelium-independent vasodilator sodium nitroprusside (maximal absolute value [% change in ratio from baseline] 600 mg allopurinol 10.11±0.66 [257.02±21.83] versus 300 mg allopurinol 9.84±0.70 [293.50±24.03] versus placebo 9.72±0.90 [309.38±28.69] mL/min per 100 mL forearm tissue; P=0.817) (Figures 1 and 2).

When vitamin C was coinfused with acetylcholine 50 and 100 nmol/min acetylcholine, the results showed that there was an improvement in flow compared with acetylcholine alone during placebo (P=0.005) and 300 mg allopurinol (P<0.001) but not with the 600-mg dose of allopurinol (P=0.36) (Figure 3).

When vitamin C was coinfused, the maximal absolute value (% change in ratio from baseline) for 600 mg allopurinol was 10.73±0.93 (346.09±61.37) versus 300 mg allopurinol 8.65±0.78 (269.40±27.22) versus placebo 5.70±0.62 (153.79±26.04) mL/min per 100 mL forearm tissue.

Figure 1. Absolute FBF data for placebo versus 300 mg allopurinol versus 600 mg allopurinol (mean±SEM). Dose 0=baseline mean. A, Acetylcholine (50, 100 nmol/min); B, sodium nitroprusside (SNP) (12.6, 37.8 nmol/min).

Figure 2. Percentage changes in FBF (corrected for noninfused arm data) for increasing doses of (A) acetylcholine (50, 100 nmol/min), (B) sodium nitroprusside (SNP) (12.6, 37.8 nmol/min) for placebo versus 300 mg allopurinol versus 600 mg allopurinol (mean±SEM).
Patients tolerated probenecid 500 mg BID very well with no withdrawals due to side effects or allergic reactions. The baseline clinical characteristics are summarized in Table 2. All patients were in sinus rhythm at the time of the study and

**Table 2.** Baseline Demographic, Hemodynamic, Humoral, and Treatment Characteristics of Patients in Study 2 (Urate Lowering With Probenecid Study)

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Value (mean ± SD)</th>
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<tbody>
<tr>
<td>Age, y</td>
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<tr>
<td>Sex (male/female), n</td>
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<tr>
<td>NYHA class (II/III), n</td>
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<tr>
<td>BMI, kg/m²</td>
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<tr>
<td>BSA, m²</td>
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<td>Systolic blood pressure, mm Hg</td>
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<td>Diastolic blood pressure, mm Hg</td>
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<td>Mean arterial pressure, mm Hg</td>
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<td>Past medical history, n</td>
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<tr>
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<td>Hypercholesterolemia</td>
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<tr>
<td>Previous myocardial infarction</td>
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<td>Smoking (current/ex/non)</td>
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<tr>
<td>Alcohol (units/week)</td>
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<td>Electrocardiogram</td>
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<td>Left ventricular hypertrophy</td>
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<td>Left bundle-branch block</td>
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<td>Medications, n (%)</td>
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<td>ACE inhibitors</td>
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<td>Angiotensin receptor blockers</td>
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<td>Aspirin</td>
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<td>β-Blockers</td>
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<td>Digoxin</td>
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<tr>
<td>Thiazide diuretics</td>
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<tr>
<td>Loop diuretics</td>
<td>12 (46)</td>
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<tr>
<td>Spironolactone</td>
<td>5 (19)</td>
</tr>
<tr>
<td>Statin</td>
<td>20 (77)</td>
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<td>Serum urea, mg/dL</td>
<td>20.7 ± 6.2</td>
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<td>Serum creatinine, mg/dL</td>
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<tr>
<td>Serum urate, mg/dL</td>
<td>7.12 ± 1.69</td>
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<tr>
<td>Serum total cholesterol, mg/dL</td>
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<td>Serum HDL, mg/dL</td>
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<td>C-reactive protein, mg/L</td>
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<td>Serum magnesium, mEq/L</td>
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<td>Plasma random glucose, mg/dL</td>
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<tr>
<td>Diabetics</td>
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<td>Nondiabetics</td>
<td>96.36 ± 12.73</td>
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<tr>
<td>HBA1c of diabetics, %</td>
<td>8.8 ± 1.7</td>
</tr>
</tbody>
</table>

Values are given as number of patients, number of medications (percentage of patients), or mean ± SD.

**Study 2: Urate Lowering With Probenecid Study**

Patients tolerated probenecid 500 mg BID very well with no withdrawals due to side effects or allergic reactions. The baseline clinical characteristics are summarized in Table 2. All patients were in sinus rhythm at the time of the study and...
all had evidence of mild to moderate LV systolic dysfunction documented by echocardiography. The underlying cause of CHF was ischemic heart disease in 25 subjects. One subject had CHF secondary to previous alcoholic cardiomyopathy.

Biochemical Parameters
Probenecid 1000 mg reduced uric acid to similar levels as allopurinol 300 mg \( (P=0.88) \) and significantly more than placebo (placebo 0.44±0.10 mmol/L; probenecid 1000 mg 0.25±0.07, \( P<0.001 \); allopurinol 300 mg 0.23 mmol/L). There were no significant differences in serum urea, creatinine clearance, plasma PIIINP, total cholesterol, HDL, CRP, insulin, and glucose among all 3 treatment periods. For BNP analysis, we subdivided the patients according to NYHA class. There was no difference in BNP levels among all 3 treatment periods for patients within each NHYA class and between NYHA classes.

Basal Flows, Washouts, and Blood Pressure
Baseline FBF between placebo and 1000 mg probenecid was not significantly different (1.78±0.10 versus 1.93±0.21; \( P=0.50 \)). There was also no significant difference between all the washout periods between the infusions for all 3 treatment periods. For each treatment phase, there was also no significant difference between the baseline and washout FBFs. Systemic blood pressures did not differ between baseline and washout periods.

Vascular Responses to Acetylcholine, Sodium Nitroprusside, and Vitamin C Coinfusion With Acetylcholine
There were no improvements seen in endothelium-dependent vasodilation in the probenecid treatment period compared with placebo (\( P=0.721 \)) (maximal absolute value [% change in ratio from baseline] 1000 mg probenecid 4.46±0.68 [99.63±20.90] versus placebo 4.67±0.65 [113.09±21.18] mL/min per 100 mL forearm tissue) (see Figure 4).

The mean flow response to acetylcholine for placebo and 1000 mg probenecid was 3.38±0.24 and 3.18±0.24 mL/min per 100 mL forearm tissue, respectively (\( P=0.70 \)). The same was seen when the data from the noninfused arm were used (\( P=0.63 \)).

There were no significant differences in the FBF responses to the endothelium-independent vasodilator sodium nitroprusside (maximal absolute value [% change in ratio from baseline] 1000 mg probenecid 4.46±0.68 [143.98±34.84] versus placebo 3.70±0.50 [162.83±31.42] mL/min per 100 mL forearm tissue; \( P=0.835 \) (Figure 4). When vitamin C was coinfused with acetylcholine50 and 100 nmol/min acetylcholine, the results showed that there was no difference between 1000 mg probenecid and placebo (\( P=0.84 \)).

Discussion
Despite having heart failure and being on loop diuretic therapy, most of the patients studied had urate levels within normal limits, which is consistent with our previously published findings.\(^1\) Nevertheless, XO inhibition resulted in further significant improvements to endothelial function. In fact, it is worth noting that increasing the dose from 300 to 600 mg improved endothelial function by 52%, whereas the extra reduction in urate was relatively small (17%). Looked at in another way, there was a 43% improvement in flow per 0.1-mmol/L fall in urate between placebo and 300 mg allopurinol. However, between 300 and 600 mg allopurinol, there was a 129% improvement in flow per 0.1-mmol/L fall in urate.

Vitamin C is a potent water-soluble scavenger of free radicals. It also reduces monocyte adhesion to endothelial cells, inhibits LDL oxidation, decreases inactivation of NO, and stimulates eNOS activity via l-arginine regeneration.\(^21\) It augments the endothelial response to intra-arterial acetylcholine. Ultra-high vitamin C was used here as a pharmacological probe to assess vascular oxidative stress in vivo in man. This is a well-accepted and established technique.\(^22–24\)
rationale is that, because the beneficial effect of vitamin C on endothelial function is due to its effect of reducing oxidative stress, this effect of vitamin C would no longer occur if the patient coincidentally received another therapy (allopurinol), which also reduced vascular oxidative stress. Our study demonstrates that high-dose allopurinol is a very effective antioxidant in the vasculature because it abolishes the vitamin C–sensitive component of oxidative stress on vascular endothelial function—ie, in the presence of high-dose allopurinol, insufficient vascular oxidative stress is formed for vitamin C to neutralize the oxidative stress and further improve endothelial function.

We used a supraphysiological dose of vitamin C (25 mg/mL). This is the standard high dose used when vitamin C is employed as a pharmacological probe.25–33 We were able to do this because the nature of the test allowed us to isolate a short stretch of artery, and therefore we were able to produce very high local concentrations. Thus we can say that allopurinol markedly reduced oxidative stress and, at the 600-mg dose, abolished oxidative stress that would have been sensitive to the high dose of vitamin C that we used.

Although our cohort of patients tolerated the high-dose allopurinol without any adverse events, we must stress the importance of patient selection with regard to renal function and close monitoring, as previous studies of the pharmacodynamics of allopurinol have shown large intersubject variability. Therefore there is still a risk of allopurinol-induced toxicity in patients with reduced renal function due to higher plasma oxypurinol concentrations.34 Another study in healthy elderly patients found that oxypurinol (but not allopurinol) clearance is reduced compared with younger subjects.35 More studies are required to assess this risk fully. Our study is, at least, very encouraging, even if the number studied is too small to be fully reassuring on safety. However, as allopurinol can be given up to a dose of 900 mg/d, we cannot be certain that even higher doses would not improve endothelial function to a greater extent.

The therapeutic implications of our study are potentially interesting because the doses used in virtually all the previous literature on XO inhibitors have fallen short of the optimum dose we found. In the LA PLATA study,36 600 mg/d oxypurinol resulted in a urate reduction of 0.10 mmol/L, which was lower than our low dose of 300 mg allopurinol (0.19 mmol/L reduction) and much lower than our 600-mg allopurinol dose (0.26-mmol/L reduction). If urate levels are used as an indicator of the degree of XO inhibition, then this suggests strongly that 600 mg/d allopurinol is more effective than the equivalent dose of oxypurinol that was used in the oxypurinol studies. We are only aware of one other clinical trial assessing endothelial function, which used 600 mg allopurinol.4 In this study, Guthikonda et al found that a single dose of 600 mg allopurinol improved endothelial function acutely in heavy smokers.

An alternative explanation is worth considering for our data on 600 mg allopurinol and vitamin C. It could be argued that the dose of vitamin C was too weak compared to the 600-mg allopurinol dose. However, this cannot be said for the 300-mg dose, and yet the same directional change was seen (a reduction in the endothelial effect of vitamin C). Furthermore, our results are entirely consistent with the known pharmacology of allopurinol and vitamin C, whereby high-dose allopurinol would reduce the production of superoxide anions such that further coinfusion of vitamin C would not encounter superoxide anions to scavenge. Overall, therefore, these known pharmacological effects seem to be the most likely explanations of our results.

The therapeutic role of allopurinol should now be reevaluated using the equivalent dose of at least 600 mg allopurinol so that we do not prematurely discard these drugs as therapeutic agents. This is even more important because the magnitude of effect on endothelial function that we have shown here with this high dose is much greater than other previously investigated therapeutic agents including angiotensin-converting enzyme (ACE) inhibitors, statins, or spironolactone. These agents have been shown to improve the vasodilatory response to acetylcholine by between 50% and 90% at best, whereas we recorded a 143% improvement with this high dose of allopurinol. This was despite the fact that 90% of our patients were already on either an ACE inhibitor or angiotensin receptor blocker and 80% on a statin (Table 1), treatments that have previously been shown to improve endothelial function.

Some studies have shown improved cardiac function and structure after long-term allopurinol treatment in CHF patients.37 The primary effect of allopurinol could be the improvement in endothelial function that has resulted in better myocardial perfusion, thereby improving LV function. Alternatively, the improvement in endothelial function could be secondary to a direct effect of allopurinol on LV function through improvement in myocardial energetic efficiency and oxygen consumption.38 Interestingly, even if improving LV function did contribute to improved endothelial function, the underlying mechanism could still be due to oxidative stress reduction as oxidative stress is a known mediator of adverse LV remodeling.

The data from our second study suggest that urate per se is probably not the culprit molecule. This further strengthens the data from the first study of an antioxidant effect of allopurinol. For comparison, 300 mg allopurinol reduced urate by 44%, whereas 1000 mg probenecid reduced urate by 46%. Despite this, 300 mg allopurinol improved endothelial-dependent vasodilatation by 52% compared with placebo, whereas probenecid at an equivalent urate-reducing dose did not alter endothelial-dependent vasodilatation at all. The main assumption in the second study is that probenecid does not have vascular effects beyond urate lowering. This is a reasonable assumption as indeed no evidence suggests otherwise.

Limitations

Our data do not discount completely the potential for urate to have an impact on endothelial function, positive or otherwise. We found that a 44% reduction in urate per se does not alter endothelial function, but greater reductions in urate could, in theory, mediate further effects. Alternatively, it is possible that urate infusions produce supraphysiological levels of urate that might alter endothelial function. Our study, however, addressed the different question of whether the fall in
urate seen with allopurinol treatment mediates its beneficial effect on endothelial function. We have not been able to obtain exact LV ejection fraction values as they are not routinely reported by the echocardiogram department at our institution. We routinely rely on a global impression of the LV systolic function. However, all patients had definite, documented, mild to moderate LV systolic dysfunction on echocardiography performed by a skilled, trained echocardiographer; were clinically diagnosed with CHF; and were being followed up in specialist heart failure clinics from which they were recruited. For the first study, 18 patients were in NYHA class II and 12 patients were in NYHA class III; in the second study, 20 patients were in NYHA class II and 6 patients were in NYHA class III.

**Conclusion**

This study shows for the first time that the mechanism of improvement in endothelial function with allopurinol lies in its ability to reduce vascular oxidative stress and not in its ability to reduce urate. Indeed, the component of endothelial oxidative stress, which was sensitive to the high dose of vitamin C we used, appears to be completely abolished by the high dose of allopurinol. We have also shown for the first time that there is a steep dose–response relationship such that allopurinol 600 mg/d improves endothelial function (143% increase compared with placebo) more than any previously seen therapy.

The main implication of our study is that, because cardiovascular events are often preceded by endothelial dysfunction and the latter can be markedly improved by high-dose allopurinol, future studies should now explore whether high-dose allopurinol will in practice reduce or limit cardiovascular events in high-risk patients.

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**Disclosures**

None.

**References**


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CLINICAL PERSPECTIVE

Allopurinol was licensed by the US Food and Drug Administration in 1966. Since then, it has been used primarily to treat patients with gout. Growing evidence indicates that it also has vascular benefits. However, there is also considerable debate as to the precise mechanism by which allopurinol improves vascular function. We performed 2 studies on patients with chronic heart failure to answer these questions. There were 3 important findings from our studies. First, we have shown that a clear and steep dose–response relationship exists between allopurinol and endothelial function, such that 600 mg/d is much more effective than 300 mg/d allopurinol. Second, for the same degree of urate lowering as allopurinol, the uricosuric agent probenecid did not produce any improvement in endothelial function. Finally, we found that high-dose allopurinol very effectively reduced oxidative stress, abolishing the added effects of high-dose vitamin C when it was coinfused. Further work is now required to determine whether high-dose allopurinol does indeed reduce vascular events.
High-Dose Allopurinol Improves Endothelial Function by Profoundly Reducing Vascular Oxidative Stress and Not by Lowering Uric Acid
Jacob George, Elaine Carr, Justine Davies, J.J.F. Belch and Allan Struthers

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