

Mechanistic Insights Into the Therapeutic Use of High-Dose Allopurinol in Angina Pectoris

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- Objectives** The aim of this study was to evaluate the effect of high-dose allopurinol on vascular oxidative stress (OS) and endothelial function in subjects with stable coronary artery disease (CAD).
- Background** Allopurinol, a xanthine oxidase inhibitor, prolongs the time to chest pain during exercise in angina. We sought to ascertain whether allopurinol also improves endothelial dysfunction in optimally treated CAD patients, because such an effect might be of value to reduce future cardiovascular mortality. The mechanism of the anti-ischemic effect of allopurinol could be related to its reducing xanthine oxidase-induced OS, and our second aim was to see whether allopurinol really does reduce vascular tissue OS in CAD patients.
- Methods** A randomized, double-blind, placebo-controlled, crossover study was conducted in 80 patients with CAD, comparing allopurinol (600 mg/day) with placebo. Endothelial function was assessed by forearm venous occlusion plethysmography, flow-mediated dilation, and pulse wave analysis. Vascular OS was assessed by intra-arterial co-infusion of vitamin C and acetylcholine.
- Results** Compared with placebo, allopurinol significantly improved endothelium-dependent vasodilation, by both forearm venous occlusion plethysmography ($93 \pm 67\%$ vs. $145 \pm 106\%$, $p = 0.006$) and flow-mediated dilation ($4.2 \pm 1.8\%$ vs. $5.4 \pm 1.7\%$, $p < 0.001$). Vascular oxidative stress was completely abolished by allopurinol. Central augmentation index improved significantly with allopurinol ($2.6 \pm 7.0\%$, $p < 0.001$) but not with placebo.
- Conclusions** Our study demonstrates that, in optimally treated CAD patients, high-dose allopurinol profoundly reduces vascular tissue OS and improves 3 different measures of vascular/endothelial dysfunction. The former effect on OS might underpin the anti-ischemic effect of allopurinol in CAD. Both effects (on OS and endothelial dysfunction) increase the likelihood that high-dose allopurinol might reduce future cardiovascular mortality in CAD, over and above existing optimum therapy. (Exploring the therapeutic potential of xanthine oxidase inhibitor allopurinol in angina; [ISRCTN15253766](#)) (J Am Coll Cardiol 2011;58:820-8) © 2011 by the American College of Cardiology Foundation

High-dose allopurinol has recently been shown to markedly prolong the time to chest pain and to ST-segment depression during exercise in patients with chronic stable angina (1). This might be related to previous experimental work suggesting that allopurinol somehow reduces myocardial oxygen consumption for a given stroke volume (2-4). Two key questions naturally arise from this. First, what is the mechanism in man whereby allopurinol delays chest pain during exercise? Second, will this anti-ischemic symptomatic benefit translate

into allopurinol also improving survival in angina? Our study was designed to shed light on both of these key questions.

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With regard to the first question, work in experimental heart failure shows that the main xanthine oxidase (XO)-related factor that influences myocardial oxygen consumption is oxidative stress (OS) (5). If allopurinol really does reduce XO-induced OS in human angina, then such an effect could well contribute to its anti-chest pain effect (6). Yet, there are currently no data (in animals or man) on whether allopurinol really does reduce OS in coronary artery disease (CAD). Furthermore, even if it does usually reduce OS, does it still do so in the presence of full contemporary CAD therapy.

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With regard to the second question posed, we also sought to gain insight into whether allopurinol might also one day improve survival in CAD. Overall survival is not usually improved in angina by treatments that improve symptoms (e.g., nitrates, amlodipine, angioplasty), aside from beta-blockers. Improved survival in CAD is usually produced instead by drugs that reduce plaque formation and instability by multiple mechanisms, including by reducing endothelial dysfunction (ED) (e.g., statins and angiotensin-converting enzyme [ACE] inhibitors). We therefore sought also to see whether allopurinol improved ED in optimally treated CAD patients to gain insight into whether there is any prospect that allopurinol might also improve survival in CAD.

One particularly relevant issue here is the contemporary treatment that CAD patients nowadays receive. This is because there is a strong possibility that treatments like statins and ACE inhibitors might already have so improved ED that targeting XO to further improve ED could be futile (7–10). As an example of this possibility, the negative results of the PEACE (Prevention of Events with Angiotensin Converting

Enzyme Inhibition) trial might have been because the ED of patients had been so improved already by statin therapy as to make ACE inhibitors ineffective. Therefore, a novel feature of our study was that we studied only optimally treated CAD patients to see whether XO inhibition can still improve ED and XO-induced OS in the presence of optimal CAD co-treatment. Only if allopurinol gives added value over contemporary treatment is it likely to be of practical therapeutic use.

Methods

Study population. Ninety consecutive patients with angiographically proven CAD (luminal stenosis of at least 50%) and preserved left ventricular systolic function (determined by either echocardiogram or left ventriculogram) were identified from cardiology clinics and an angiogram database within a period of just <12 months. Baseline characteristics are noted in Table 1. Single vessel disease was present in 17 of 80, double vessel disease was present in 30 of 80, and triple vessel disease was present in 33 of 80. Approximately 85% of the subjects had undergone successful revascularization in the past. However, coronary revascularization had obviously not directly altered the arm blood vessels we were studying. The following were exclusion criteria: unstable angina (last 3 months), cardiac failure, malignancy, use of warfarin or allopurinol, chronic kidney disease stage 4 or worse, hypertension (systolic blood pressure >160 mm Hg and diastolic blood pressure >100 mm Hg), and allopurinol allergy. All patients provided written informed consent, and the study was approved by the Tayside committee on medical research ethics. The study was conducted in accordance with the Declaration of Helsinki.

Study protocol. This was a randomized, double-blind, placebo-controlled, crossover trial, with a 4-week washout between treatments (Fig. 1). After initial screening, subjects were randomized to receive either allopurinol (Ranbaxy Ireland, Ltd., Tipperary, Ireland) or matching placebo (Penn Pharmaceutical Services, Tredegar, Wales) for 8 weeks. Randomization was done by using an Internet program, and subjects were randomized to receive either treatment A or B (allopurinol or placebo) first. Randomization code was kept sealed in the department. Investigators and patients were blinded to the order of the treatment they received. The dose of allopurinol was 300 mg for 4 weeks and 600 mg for 4 weeks with a 4-weekly check on hematology and biochemistry.

Abbreviations and Acronyms

ACE	= angiotensin-converting enzyme
Ach	= acetylcholine
Aix	= augmentation index
CAD	= coronary artery disease
CI	= confidence interval
CV	= cardiovascular
ED	= endothelial dysfunction
FBFR	= forearm blood flow ratio
FMD	= flow-mediated dilation
NO	= nitric oxide
OS	= oxidative stress
Ox-LDL	= oxidized low-density lipoprotein
SNP	= sodium nitroprusside
XO	= xanthine oxidase

Table 1 Baseline Characteristics of Subjects

Total no. of subjects (M/F)	80 (64/16)
Age (yrs)	65.5 ± 7.73
No. of vessels diseased (1/2/3)	17/30/33
CCS class (1/2/3)	58/22/0
Medical treatment/PCI/CABG	11/47/22
Current smoker/ex-smoker/nonsmoker	11/26/43
Past medical history	
Hypertension	31 (39)
Hypercholesterolemia	58 (74)
Previous myocardial infarction	21 (26)
Diabetes mellitus	7 (9)
Medications	
ASA/clopidogrel	80 (100)
ACE inhibitor/ARB	68 (85)
Ramipril equivalent dose, average (mg)	7.35
Beta-blockers	60 (75)
Statins	76 (95)
Simvastatin equivalent dose, average (mg)	37
Thiazide diuretics	9 (11)
Nitrate	21 (26)
Biochemical and hemodynamic data	
Na (mmol/l)	141 ± 2.1
K (mmol/l)	4.5 ± 0.3
Urea (mg/dl)	16.8 ± 4.2
Creatinine (mg/dl)	1.08 ± 0.15
Total cholesterol (mg/dl)	150.8 ± 31.5
Creatinine clearance (ml/min)	78 ± 25
Heart rate (beats/min)	61 ± 10
SBP (mm Hg)	132 ± 12
DBP (mm Hg)	75 ± 8
MAP (mm Hg)	93 ± 8

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

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; ASA = acetylsalicylic acid; CABG = coronary artery bypass graft surgery; CCS = Canadian Cardiovascular Society; DBP = diastolic blood pressure; K = potassium; MAP = mean arterial pressure; Na = sodium; PCI = percutaneous coronary intervention; SBP = systolic blood pressure.

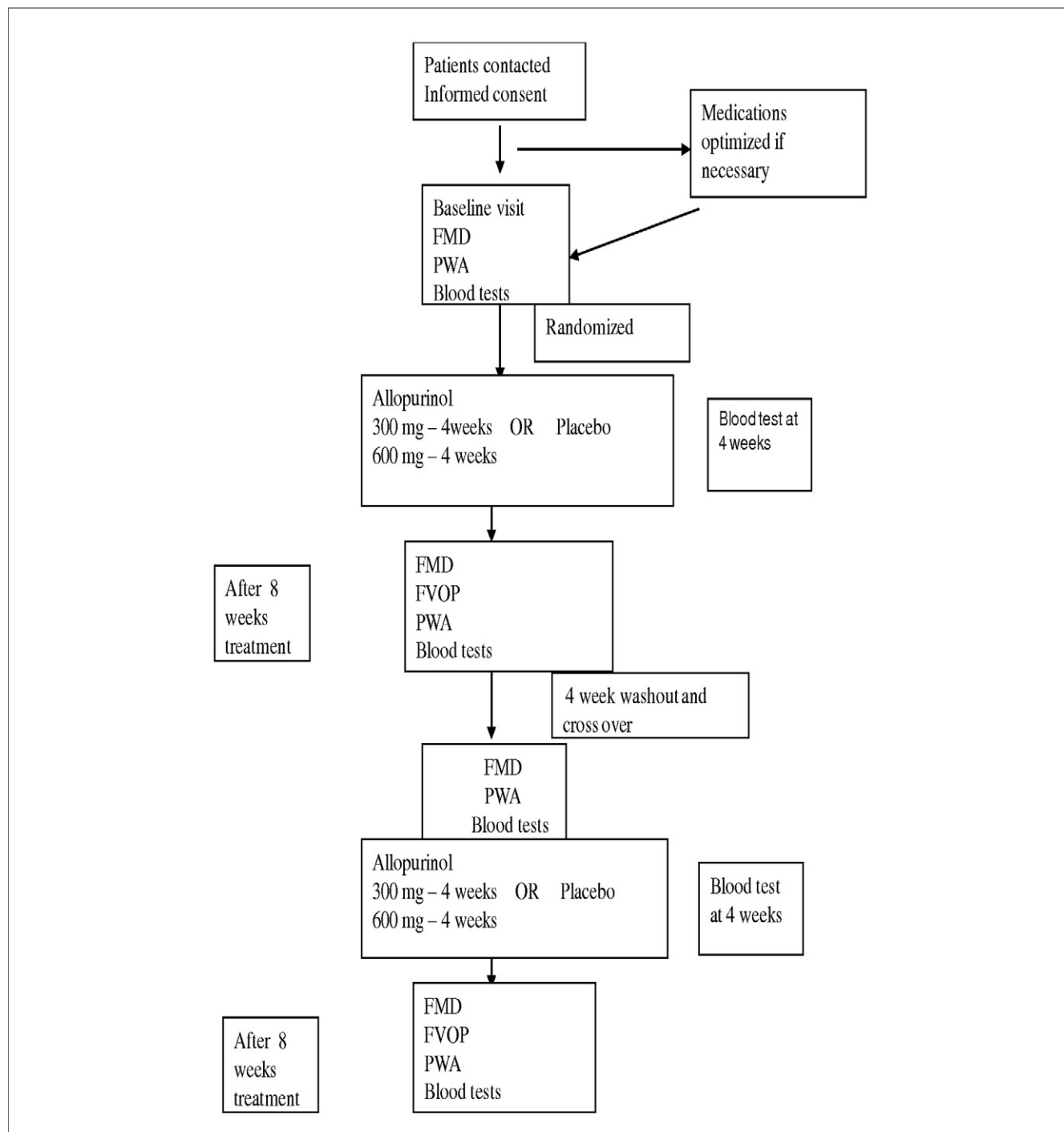


Figure 1 Study Design

Schematic representation of study design and flow. FMD = flow-mediated dilation; FVOP = forearm venous occlusion plethysmography; PWA = pulse wave analysis.

Forearm venous occlusion plethysmography. Forearm venous occlusion plethysmography was performed at the end of each treatment, and our protocol has been published previously (6,11,12). Acetylcholine (Ach) (25, 50, and 100 nmol/min, Novartis, Basel, Switzerland) was first infused, followed after washout by sodium nitroprusside (SNP) (Mayne Pharma, Leamington, Warks, United Kingdom) at 37.8 nmol/min.

Thereafter, to study OS, vitamin C 25 mg/ml (ascorbic acid, UCB Pharma, Ltd., Slough, United Kingdom) was coinfused with Ach 25, 50, and 100 nmol/min.

Flow-mediated dilation. Flow-mediated dilation (FMD) was performed at the beginning and the end of each treatment. The FMD has been described by us previously according to international guidelines (13,14).

Augmentation index. Central augmentation index (AIx) corrected to a heart rate of 75 beats/min was determined by pulse wave analysis as previously described in 39 patients at the beginning and the end of each treatment (15). All vascular studies were performed and analyzed by a single trained investigator (N.S.R.).

Laboratory tests. Blood was collected at baseline and at the end of each treatment after 30 min of supine rest. B-type natriuretic peptide (Bachem, Ltd., St. Helens Merseyside, United Kingdom), uric acid (colorimetric assay, Cobas Bio, Roche, Lewes, United Kingdom), and procollagen 3-N terminal peptide (radioimmunoassay, Oxford Biosystems, Oxford, United Kingdom) were analyzed at the end of the study. Oxidized low-density lipoprotein (Ox-LDL) levels were measured by a Mercodia Ox-LDL enzyme-linked immunoadsorbent assay kit, and plasma levels of F2-isoprostanes were measured by gas chromatography–mass spectrometry. We standardized to the isoprostane 8-epi-PGF2-alpha with 2,4-tetra-D2-8-epiprostaglandin F2-alpha as internal standard.

Statistical analysis. The sample size was based on a mean forearm blood flow ratio (FBFR) of 263 when Ach 100 was infused, as seen previously by us. To detect a 20% change with an SD of difference of 166, we needed 79 completers to have 80% power at $p < 0.05$.

Data were analyzed for normal distribution and presented as mean \pm SD. Data were analyzed for any carryover effect by comparing the baselines and for sequence effect by testing for interaction of on-treatment change. Paired *t* test or Wilcoxon signed rank test was used as appropriate. Forearm blood flow values (expressed as ml/min/100 ml forearm tissue) were converted to ratio (the ratio of increase in blood flow in infused arm to control arm, FBFR) and percentage increase in FBFR from preceding baseline (absolute ratio). The data were analyzed by repeated measures analysis of variance with Bonferroni method for calculating 95% confidence interval (CI), with treatment and order of infusion as fixed factors, and patient number as the random factor. A 2-tailed *p* value of <0.05 was the criterion for statistical significance. Analyses were carried out with SPSS for Windows (version 12.0, SPSS, Inc., Chicago, Illinois).

Table 2 Effect of Allopurinol and Placebo on Endothelial Function, Serum Urate, and Serum Biomarkers

Parameter, n	Allopurinol	Placebo	p Value
FMD, % (n = 76)	5.4 \pm 1.7	4.1 \pm 1.8	<0.001
AIx, % (n = 39)	24.7 \pm 4.6	27.6 \pm 5.5	<0.001
Urate, mg/dl (n = 77)	2.54 \pm 0.84	5.76 \pm 1.18	<0.001
BNP, pg/dl (n = 74)	52.8 \pm 2.3	69.3 \pm 2.5	NS
PIIINP, mg/l (n = 72)	3.44 (0.97)	3.35 (0.88)	NS
CrCl, ml/min	83 \pm 28	82 \pm 27	NS
Ox-LDL, U/l	48.9 \pm 1.8	57.3 \pm 4	0.01
F2-Isoprostanes, pg/ml	240 \pm 93	259 \pm 113	0.09

AIx = augmentation index; BNP = B-type natriuretic peptide; CrCl = creatinine clearance; FMD = flow-mediated dilation; Ox-LDL = oxidized low-density lipoprotein; PIIINP = procollagen 3-N terminal peptide.

Results

Of the 90 patients recruited, 80 completed the study (Table 1). Allopurinol was well tolerated with no adverse events reported. In particular, there were no reports of rash or worsening renal function (Table 2). Six patients dropped out, citing personal reasons; 1 was admitted with troponin negative angina (taking allopurinol); 2 gave no reason; and 1 patient dropped out, due to generalized malaise, while taking placebo.

Forearm venous occlusion plethysmography. BASAL FLOWS, WASHOUTS, AND BLOOD PRESSURE. Baseline FBFR did not differ between allopurinol and placebo ($p = 0.493$). Also, no significant difference in FBFR was apparent for washouts after infusing Ach and SNP either within a treatment phase or between allopurinol and placebo. Systemic blood pressure and heart rate did not vary between the baseline and washout periods.

RESPONSE TO ACH, SNP, AND VITAMIN C COINFUSION WITH ACH. There was a significant 52% increase in endothelium-dependent vasodilation with allopurinol, compared with placebo ($p = 0.006$) (Fig. 2).

When vitamin C was coinfused with Ach, there was a highly significant increase in FBFR only during placebo ($p < 0.001$) but not during allopurinol ($p = 0.386$) (Fig. 3).

FMD. Allopurinol increased brachial artery FMD highly significantly from a baseline of $4.2 \pm 1.8\%$ to $5.4 \pm 1.7\%$ (95% CI: 1.18 to 1.42, $p < 0.001$), whereas placebo did not produce any change ($4.2 \pm 1.8\%$ to $4.1 \pm 1.8\%$, 95% CI: -1.12 to 1.08 , $p = 0.76$) (Fig. 4).

AIx. Allopurinol significantly reduced central AIx corrected to heart rate of 75 beats/min, from $27.3 \pm 5.0\%$ to $24.7 \pm 4.6\%$ (95% CI: 0.41 to 4.84, $p < 0.001$), whereas there was no significant change with placebo ($27.8 \pm 5.9\%$ to $27.6 \pm 5.5\%$, 95% CI: -2.4 to 2.64 , $p = 1.0$) (Fig. 5).

Biochemistry and biomarkers. Allopurinol significantly reduced Ox-LDL levels (57.3 ± 4 U/l vs. 48.9 ± 1.8 U/l; $p = 0.01$) and was associated with lower levels of F2-Isoprostanes, compared with placebo, although this failed to reach statistical significance (240 ± 93 pg/ml vs. 259 ± 113 pg/ml; $p = 0.09$) (Table 2). B-type natriuretic peptide levels did fall on allopurinol, although the results fell short of statistical significance.

Discussion

This study has 3 main findings. The first is that, despite full optimum therapy, CAD patients still exhibit marked vascular OS (i.e., intra-arterial high-dose vitamin C improves vascular responses to Ach by 100% during placebo). Our second finding is that endogenous XO is a major source of this vascular tissue OS as well as of widespread vascular dysfunction. Our third finding is that XO inhibition profoundly improves both vascular tissue OS and widespread vascular dysfunction in CAD patients who are already taking optimal CAD therapy.

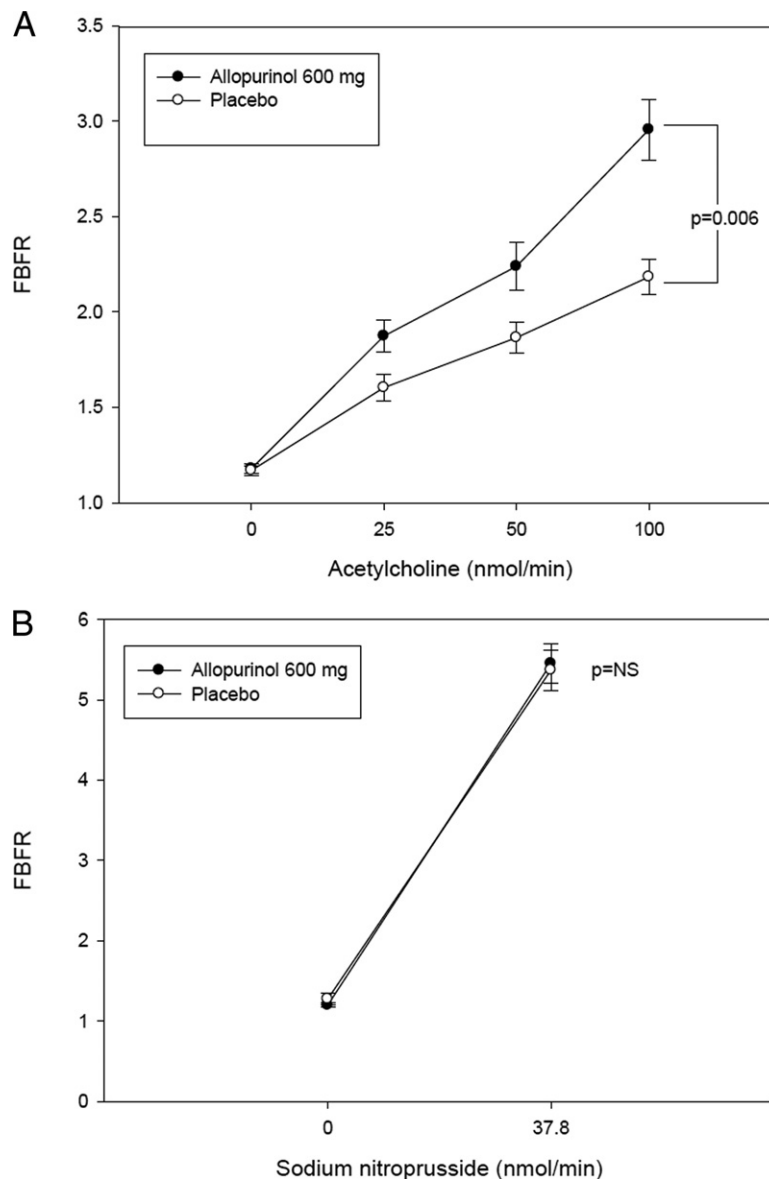


Figure 2 Endothelial Function

Forearm blood flow ratio (FBFR) data for placebo versus allopurinol (mean \pm SEM).

(A) Acetylcholine (25, 50, and 100 nmol/min); (B) sodium nitroprusside (37.8 nmol/min).

The clinical implications of our results are 2-fold. Firstly, because XO-induced OS is associated with increased myocardial oxygen consumption in animal studies, our finding that allopurinol profoundly decreases XO-induced OS in treated CAD patients could well contribute mechanistically to the recently observed anti-ischemic effect of allopurinol in patients with stable angina (1). Part of the reason for this could be that molecular oxygen is a substrate for XO, meaning that endogenous XO activity effectively reduces the supply of oxygen to ischemic tissue (16). In fact, XO is known to consume a lot of oxygen not only because it produces

superoxide anions and hydrogen peroxide but also because the main XO substrate (hypoxanthine) has only 1 oxygen/molecule, whereas the main XO product, uric acid, has 3. Thus, XO-induced OS might not be a culprit per se but instead could be a marker that precious molecular oxygen is being “wasted” (by XO) in producing OS. If this were the case, this might also be a novel explanation for why OS often seems to be a culprit but neutralizing OS with antioxidant vitamins has been disappointing. A whole alternative explanation, of course, is that XO-induced OS is itself a culprit, able by itself to increase tissue oxygen consumption, as some data suggest (5).

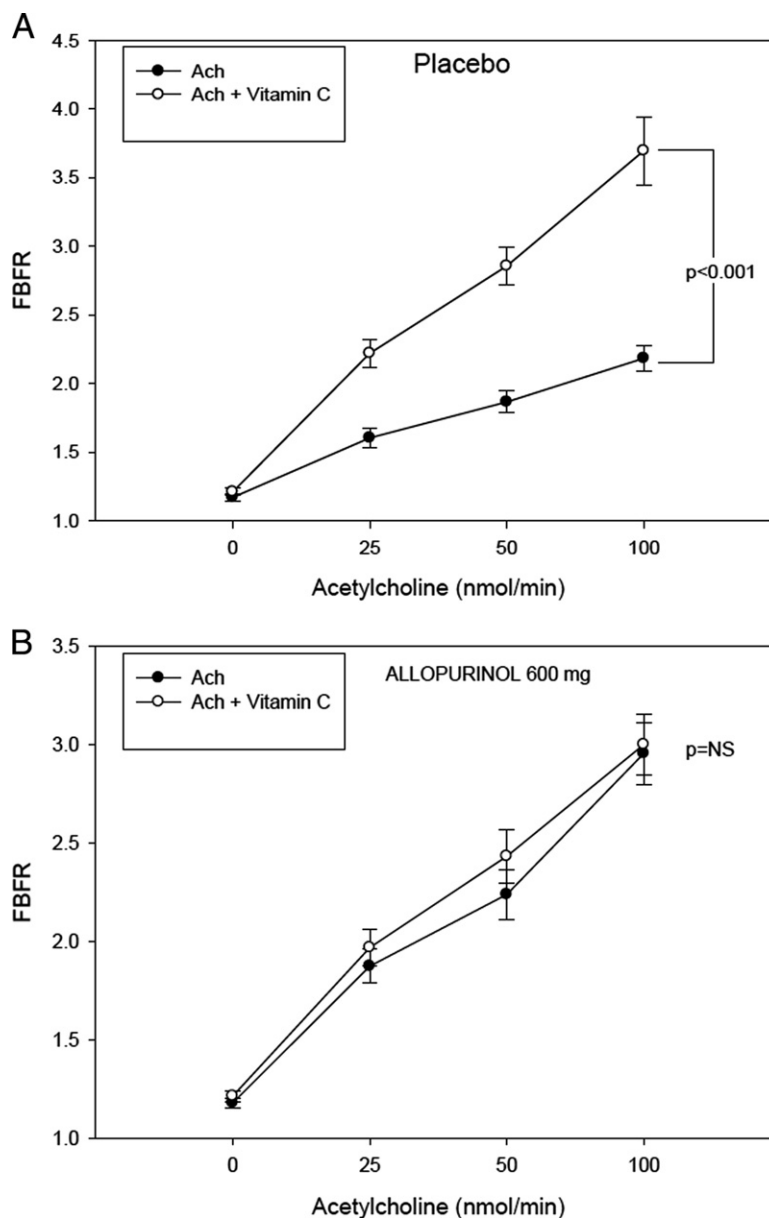
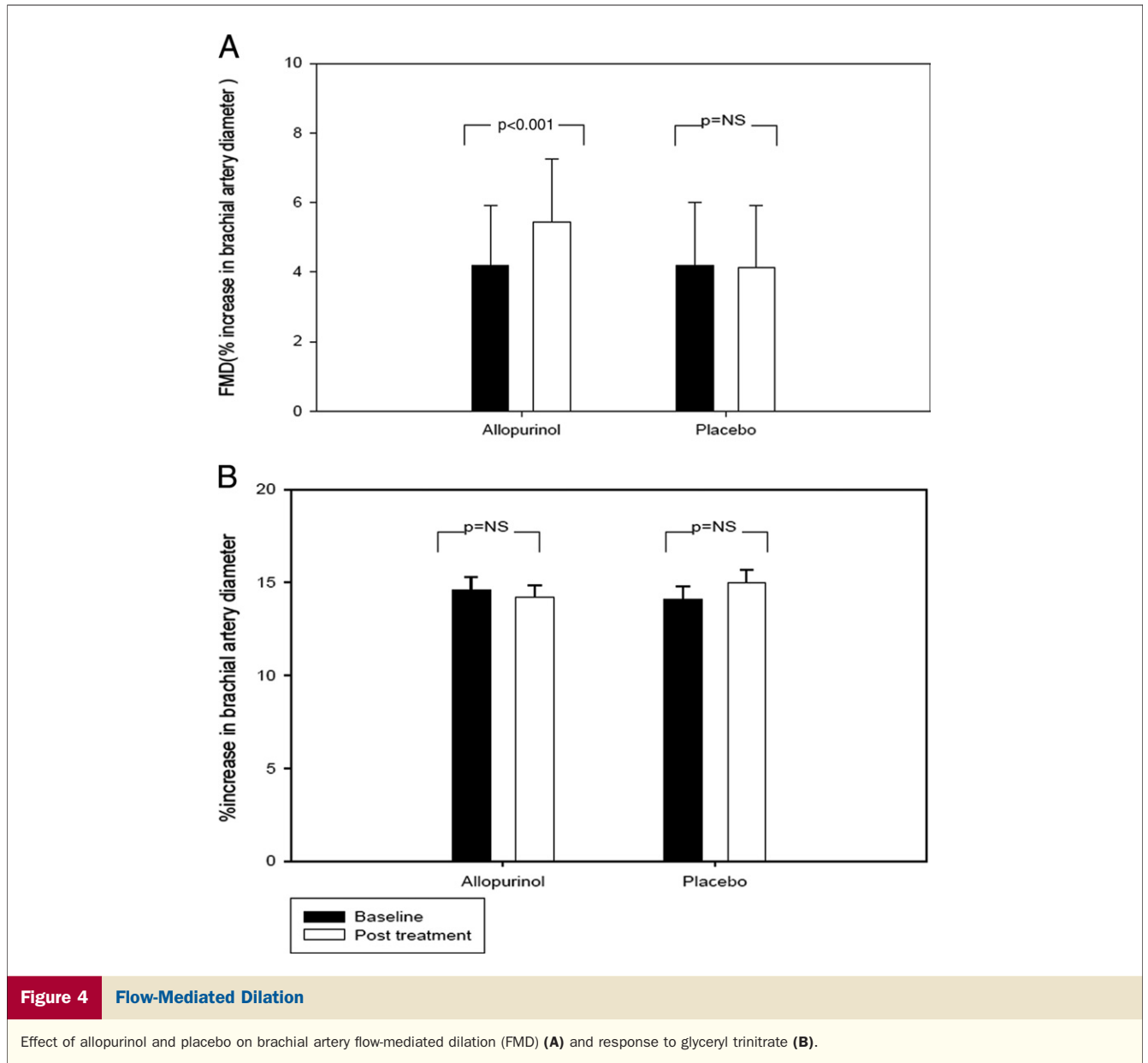


Figure 3 Vascular Oxidative Stress

Forearm blood flow ratio (FBFR) data (mean \pm SEM) for acetylcholine (25, 50, and 100 nmol/min) alone versus acetylcholine + vitamin C (25 mg/ml) for placebo (A) and allopurinol (B)—an in vivo measure of vascular oxidative stress.

The second clinical implication follows from our demonstration that allopurinol markedly improves endothelial/vascular dysfunction in CAD. This raises the separate possibility that allopurinol might improve survival in CAD. In fact, dysfunction in all 3 of our measures of endothelial/vascular function are each independently linked with prognosis, and therefore it is particularly promising that allopurinol improved all 3, although clearly not all therapies that improve endothelial function always benefit hard clinical endpoints (17–20). It is also true that no other therapy has consistently improved 3 different measures of vascular

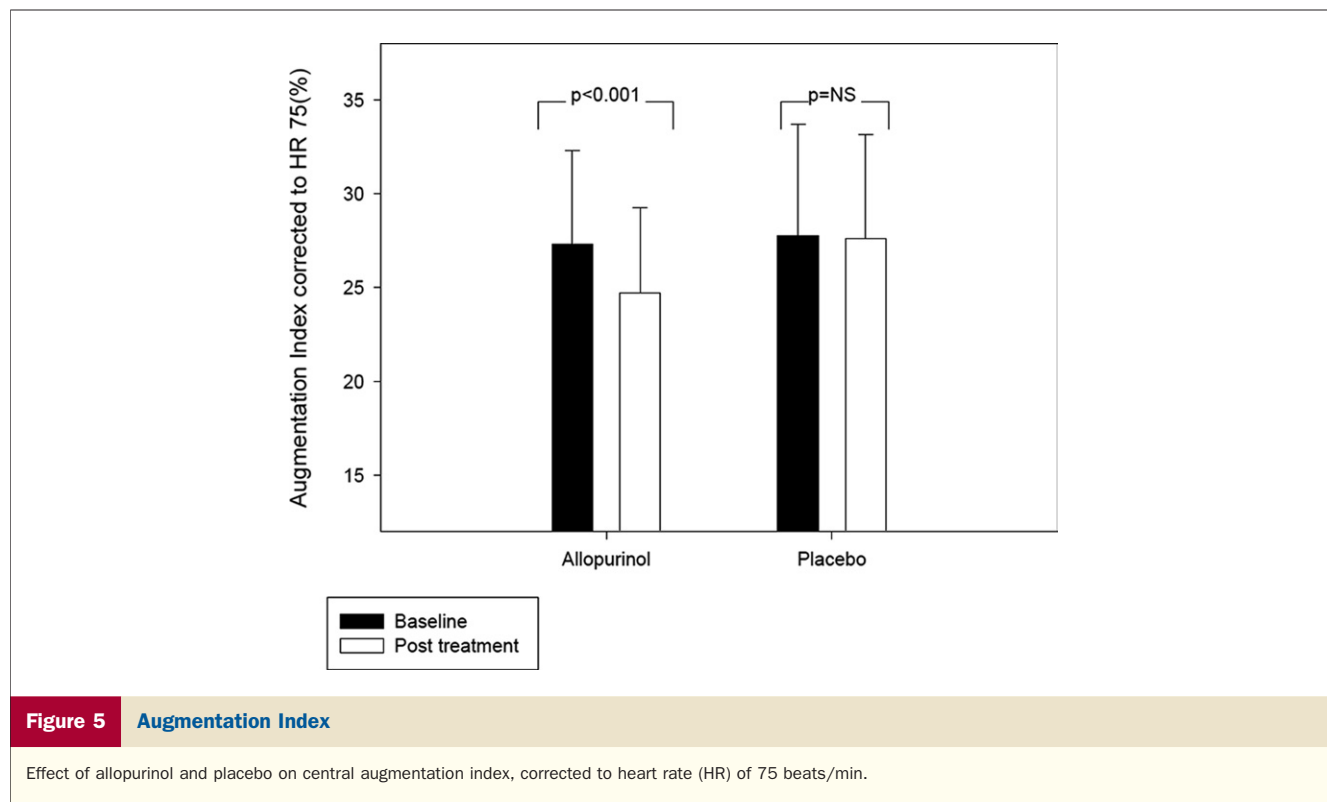
dysfunction: improvement in 1 or 2 but not 3 is common (21). Furthermore, the effect of allopurinol on OS could also be relevant here, because vascular OS—as measured by the vitamin C technique used here—is also known to predict cardiovascular (CV) events independently of endothelial function: indeed, vascular OS seems to be an even better predictor of future CV events than endothelial function per se (22). This might be because OS is thought to contribute particularly to plaque instability (23). Therefore, overall, it could turn out that allopurinol will improve both the time to chest pain during exercise and the number of future (CV)



deaths. The former has already been demonstrated (1). The latter is at this point in time only a hopeful prospect, but its likelihood is reinforced by our data on ED and OS as well as by 2 large observational studies where the use of allopurinol was associated in both studies with a highly significant reduction in total mortality (22% reduction in 1 study, and a 26% reduction in the other) (24,25). If so, allopurinol could turn out to be the only antianginal, other than beta-blockers, that improves both symptoms and survival—which would elevate allopurinol into not just being yet another antianginal.

The vitamin C technique we used here to assess vascular OS is a well-established technique (6,22,26,27) with 3 main advantages over blood or urine OS biomarkers: it measures function rather than just levels, it reflects vascular tissues rather than just blood or urine, and it is an *in vivo* measure

rather than an *ex vivo* or *in vitro* measure. Indeed, plasma or urine biomarkers for OS have their limitations in therapeutic studies (28): for example, successful therapies like statins do not produce consistent changes in very many OS biomarkers (29,30). Because we were mainly interested here in vascular OS rather than circulating OS, the vitamin C technique is the gold standard in our study. The dose of vitamin C we used (25 mg/ml) is extremely high and effectively much higher than used in any large antioxidant clinical trial. When co-infused with Ach, high-dose parenteral vitamin C enhances vasodilation, because it reduces the vascular OS that would normally degrade endogenous nitric oxide (NO). Therefore, if there were to be no OS, then vitamin C would not have any enhancing effect on Ach-mediated vasodilation, which is exactly what we found in the presence of high-dose allopurinol. An important point is that we previously saw the same large



effect on vascular OS in heart failure patients, which adds credibility to our results here (6).

A recognized disadvantage of using vitamin C in this way is the concern that the results obtained can sometimes be because the blood vessels have reached their vasodilatory ceiling, but this cannot be the case here because both SNP and the top-dose Ach/vitamin C response with placebo produced a higher absolute FBFR than when allopurinol was administered.

The magnitude of the effect against vascular tissue OS is so large here and in our previous congestive heart failure study that it becomes possible that allopurinol reduces OS by mechanisms additional to XO inhibition, although no such extra mechanism has yet been identified (6). Even if some additional mechanism is involved, this does not detract from the strong therapeutic potential for high-dose allopurinol in CAD. Another explanation for such a large effect of allopurinol here is that endogenous XO activity is up-regulated in ischemic tissue (31). A third explanation for the magnitude of the effect seen here is the high dose of allopurinol used, which incidentally matched the high dose that we found to be anti-ischemic (1).

Ox-LDL is not a unique chemical entity, and therefore different assays detect different epitopes in this complex molecule. Therefore, each assay has limitations, including ours—which mainly measures oxidized apolipoprotein-B (28). However, our Ox-LDL results are corroborated by Tsutsumi et al. (32), who found in gout that allopurinol significantly reduced Ox-LDL with a completely different assay. Other factors increase the credibility of our Ox-LDL

results. First, they agree with our main vitamin C results on vascular OS. Second, they agree with our $p = 0.09$ reduction in isoprostanes. Third, they agree with much previously published data that allopurinol reduces another OS biomarker, malondialdehyde (11,12). Fourth, Tsutsumi et al. (32) found that allopurinol reduced Ox-LDL but did not change low-density lipoprotein cholesterol itself, and it is changes in the latter that are the main confounder with our Ox-LDL assay (32). In any case, the Ox-LDL results are merely corroborative here, because our study was primarily designed to measure vascular tissue OS, which might not necessarily correlate with all plasma OS biomarkers, because each one assesses different aspects of circulating OS.

It is worth saying that it is at least plausible that the lowering of uric acid per se could have contributed directly to the improvement in endothelial function and in OS that we saw. This follows from recent observations that uric acid can activate OS in vitro (33).

A minor weakness of our study is that we were unable to assess in vivo whether the improved endothelial function was definitely due to NO or not. Neither NG-monomethyl-L-arginine nor nitrite measurements would have been informative here, because both mainly reflect NO synthesis, whereas allopurinol probably acts primarily on the inactivation of already formed NO. By contrast, our study has many strengths, such as clear-cut results for all vascular measures, the use of the gold standard invasive technique for assessing vascular tissue OS, and the magnitude of the effect seen in all vascular parameters. Another strength is that we used 3

complementary methods to assess endothelial function, whereas most studies use 1 or at most 2 such methods.

Conclusions

Despite full contemporary treatment in CAD patients, vascular OS remains marked, and endogenous XO is a major source of it. As a result, allopurinol profoundly reduces both vascular OS and 3 separate measures of ED in CAD. The former effect could underpin the recently demonstrated anti-ischemic effect of allopurinol in angina (1). These results (on OS and ED) also raise the prospect that allopurinol might reduce future CV events and deaths in CAD, especially because OS is thought to contribute to plaque instability and also because allopurinol is already associated with a significantly better total mortality in 2 large observational studies (24,25).

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