Effects of Xanthine Oxidase Inhibition With Allopurinol on Endothelial Function and Peripheral Blood Flow in Hyperuricemic Patients With Chronic Heart Failure

Results From 2 Placebo-Controlled Studies

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Background—In patients with chronic heart failure (CHF), hyperuricemia is a common finding and is associated with reduced vasodilator capacity and impaired peripheral blood flow. It has been suggested that the causal link of this association is increased xanthine oxidase (XO)–derived oxygen free radical production and endothelial dysfunction. We therefore studied the effects of XO inhibition with allopurinol on endothelial function and peripheral blood flow in CHF patients after intra-arterial infusion and after oral administration in 2 independent placebo-controlled studies.

Methods and Results—In 10 CHF patients with normal serum uric acid (UA) levels (315±42 μmol/L) and 9 patients with elevated UA (535±54 μmol/L), endothelium-dependent (acetylcholine infusion) and endothelium-independent (nitroglycerin infusion) vasodilation of the radial artery was determined. Coinfusion of allopurinol (600 μg/min) improved endothelium-dependent but not endothelium-independent vasodilation in hyperuricemic patients (P<0.05). In a double-blind, crossover design, hyperuricemic CHF patients were randomly allocated to allopurinol 300 mg/d or placebo for 1 week. In 14 patients (UA 558±21 μmol/L, range 455 to 743 μmol/L), treatment reduced UA by >120 μmol/L in all patients (mean reduction 217±15 μmol/L, P<0.0001). Compared with placebo, allopurinol improved peak blood flow (venous occlusion plethysmography) in arms (+24%, P=0.027) and legs (+23%, P=0.029). Flow-dependent flow improved by 58% in arms (P=0.011). Allantoin, a marker of oxygen free radical generation, decreased by 20% after allopurinol treatment (P<0.001). There was a direct relation between change of UA and improvement of flow-dependent flow after allopurinol treatment (r=0.63, P<0.05).

Conclusions—In hyperuricemic CHF patients, XO inhibition with allopurinol improves peripheral vasodilator capacity and blood flow both locally and systemically. (Circulation. 2002;105:2619-2624.)

Key Words: heart failure • blood flow • endothelium

Impairment of peripheral blood flow and reduced vasodilator capacity are constant findings in chronic heart failure (CHF) and relate closely to such prominent clinical symptoms as reduced exercise capacity and early muscle fatigue. 1,2 Endothelial function 3 and vasodilator reactivity to exercise 4 have been shown to be significantly reduced in patients with CHF. One major factor responsible for the impaired regulation of vascular tone in CHF is the increase in oxidative stress, leading to a premature breakdown of endothelium-derived vasoactive nitric oxide (NO). An important source for oxygen free radical production within the endothelium is the enzyme xanthine oxidoreductase (EC1.1.3.22). In its oxidase form, 5 this enzyme generates superoxide anion and hydrogen peroxide as byproducts.

It has been shown that in CHF, high serum uric acid (UA) levels reflect the degree of xanthine oxidase (XO) activation in CHF; 6 hyperuricemia occurs independently of the effects of diuretics and renal impairment. 7 In CHF patients, serum UA levels rise in parallel with NYHA functional class, and hyperuricemia predicts impaired peripheral blood flow and increased vascular resistance of the leg vascular bed. 8 Theoretically, inhibiting XO and hence reducing serum UA may improve endothelial function and vasodilator capacity in CHF.
It has been shown that allopurinol treatment can improve forearm blood flow and endothelial dysfunction in patients with type 2 diabetes mellitus and mild hypertension. In the context of reperfusion injury, it is understood that XO-derived oxygen free radicals are a major contributor to impaired flow and tissue damage and that allopurinol may exert protective effects against these reperfusion injuries. Circulating XO has been shown to contribute to vascular dysfunction in animal models of hypercholesterolemia, and some reports suggest direct damaging effects of UA. We tested the hypothesis that in patients with CHF, (1) infusion of allopurinol has an acute beneficial effect on endothelium-dependent vasodilator capacity and (2) oral treatment with allopurinol improves vasodilator capacity and peripheral blood flow in patients with chronic heart failure and hyperuricemia.

Methods
Two studies were performed in 2 independent study centers and by separate study personnel. Both studies were approved by the local ethics committees, and written informed consent was obtained from all patients. In both studies, patients with impaired left ventricular systolic function (ejection fraction <40%) were eligible for inclusion. Patients were in stable clinical condition for ≥4 weeks before enrollment, with optimized conventional medical therapy.

Study 1: Allopurinol Infusion Study

Patients
Nineteen male patients <70 years old with CHF caused by idiopathic dilated cardiomyopathy or ischemic heart disease were investigated. Exclusion criteria were any conditions potentially affecting endothelial function, ie, arterial hypertension (>160/90 mm Hg), diabetes mellitus (fasting glucose >120 mg/dL), hypercholesterolemia (total cholesterol >200 mg/dL), and current smoking. Patients were divided into 2 groups according to serum UA levels (normal UA, ≤440 μmol/L; elevated UA, >440 μmol/L).

Protocol
For intra-arterial infusions, a 20-gauge catheter was placed into the brachial artery. The internal diameter of the radial artery was determined by high-precision ultrasound as described previously. For dependent vasodilation, the brachial artery was occluded for 5 minutes. Endothelium-dependent vasodilation was assessed by use of acetylcholine (Mischol 20 mg/2 mL, Ciba-Vision) in a low dose (7.5 μg/min) and a high dose (30 μg/min) at a flow rate of 1 mL/min for 5 minutes at each concentration. For determination of flow-dependent vasodilation, the brachial artery was occluded for 5 minutes by a blood pressure cuff inflated to 50 mm Hg above the systolic blood pressure. The internal diameter of the radial artery was recorded continuously for 2 minutes in 10-second intervals after the cuff was deflated. Endothelium-independent vasodilation was determined by use of nitroglycerin (glyceroltrinitrate; Schwarz Pharma) at a dose of 0.2 mg/mL and a flow rate of 1 mL/min. This infusion protocol was applied to every patient during intra-arterial infusion with saline (flow rate 1 mL/min) and then repeated during infusion with allopurinol (600 μg/min, flow rate 1 mL/min).

Study 2: Placebo-Controlled Treatment Study

Patients
CHF patients with hyperuricemia (UA >400 μmol/L) were eligible for the study. Patients were treated with diuretics (93%), ACE inhibitor or angiotensin II antagonists (92%), β-blockers (60%), aspirin or warfarin (93%), and nitrates (33%). Exclusion criteria were a history of unstable angina, myocardial infarction or stroke within 3 months before the study, any life-threatening disease including malignancy within the previous 5 years, active myocarditis, serum creatinine >300 μmol/L, severe liver disease (ASAT or ALAT >3 times the upper limit of normal range), gout, or a history of allopurinol therapy.

Protocol
The study used a placebo-controlled, double-blind, crossover design. After the baseline visit, patients were randomly allocated to either therapy with allopurinol (300 mg/d) or matching placebo. After a treatment period of ≥7 days, each patient was switched to the respective opposite treatment option for another 7 days. The primary end point of the study was the postischemic peak leg blood flow after allopurinol therapy versus placebo. As secondary end points, changes in resting arm and leg blood flow, ischemia-stimulated peak arm blood flow, and forearm flow-dependent flow as well as serum UA levels were assessed. As tertiary end point, allantoin, a biochemical marker of oxygen free radical load, was analyzed.

Blood Sampling and Blood Flow Assessment
At each study visit, venous blood samples were taken after 15 minutes of supine rest for the assessment of serum UA levels (uricase-peroxidase method) and other routine biochemical parameters (all measured in the hospital routine system). Arm and leg blood flow (given in mL ∙ 100 mL⁻¹ ∙ min⁻¹) were determined consecutively at the right arm and leg using strain-gauge venous occlusion plethysmography (EC4, Hokanson) as described previously. Resting flow was assessed in the supine position after a resting period of ≥15 minutes. Stimulated peak blood flow during reactive hyperemia was assessed immediately after a period of total ischemia. Ischemia was induced by external proximal compression of the respective limb (30 mm Hg above the systolic blood pressure) for 3 minutes (arm) and 5 minutes (leg). After cuff deflation, blood flow was measured for 2 minutes in 10-second intervals. The highest flow result was recorded as postischemic peak blood flow. For assessment of flow-dependent flow, a second sphygmomanometer cuff was placed distal to the strain-gauge and inflated to suprasystolic levels for 2 minutes. After deflation of this cuff, flow was measured every 10 seconds for 90 seconds. The sudden increase of shear stress causes endothelium-dependent NO release. Flow-dependent flow is therefore regarded as a noninvasive method to estimate endothelium-dependent vasodilatation.

Allantoin Assessment
Allantoin results from further oxidation of UA. Because the enzyme involved, uricase, is not expressed in humans, allantoin may occur in human tissue exclusively as a result of nonenzymatic reaction with highly reactive oxygen species. Elevated levels are therefore regarded as a marker of increased oxidative stress. Allantoin was measured by gas chromatography–mass spectrometry (with a stable-isotope internal standard) after anion exchange extraction. Intra-assay imprecision was 3.75%; interassay imprecision was 12%. The lower limit of detection was 0.33 μmol/L. By this method, the normal value for healthy male subjects 45 to 70 years old is 14.9 μmol/L (D.A. Reaveley, PhD, Imperial College, London, UK, unpublished data, 2001).

Statistical Analysis
Data are presented as mean±SEM. The crossover treatment trial was analyzed according to the recommendations of Hills and Armitage for crossover trials. The primary and secondary end points were analyzed for the presence of period and carryover effects, none of which were found. Results are therefore reported for all patients together in the order baseline—treatment—placebo. Repeated-measures ANOVA and paired t test were used where appropriate. The relationship between the variables was analyzed by simple linear regression (least-squares method). A value of P<0.05 was considered significant.
TABLE 1. Allopurinol Infusion Study: Patient Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Uric Acid* (n=10)</th>
<th>Elevated Uric Acid* (n=9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58.4±3.8</td>
<td>55.8±3.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Etiology (ischemic/dilative)</td>
<td>6/4</td>
<td>5/4</td>
<td>0.8</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>23.4±2.2</td>
<td>18.33±1.9</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.8±1.2</td>
<td>26.5±0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>125±6.3</td>
<td>118±6.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>77.7±3.8</td>
<td>77.3±4.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Uric acid, μmol/L</td>
<td>315±42</td>
<td>535±54</td>
<td>0.003</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.5±0.2</td>
<td>5.7±0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>176.3±16.9</td>
<td>179.2±24.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

LVEF indicates left ventricular ejection fraction; BMI, body mass index; and BP, blood pressure.

*Normal uric acid levels, ≤440 μmol/L; elevated uric acid levels, >440 μmol/L.

Results

Study 1: Allopurinol Infusion Study

Clinical characteristics of the patients are given in Table 1. The 2 study groups with normal (315±42 μmol/L) and high (535±54 μmol/L) UA levels were similar in age and main clinical and biochemical parameters. Results from the infusion protocol are shown in Table 2. In hyperuricemic patients, infusion with allopurinol improved endothelium-dependent vasodilator capacity at high-dose acetylcholine infusion (P<0.05) and showed a strong trend at the low-dose acetylcholine infusion (P=0.06) compared with saline infusion.

Endothelium-dependent flow-dependent vasodilation also improved with allopurinol coinfusion compared with placebo (P<0.05). Endothelium-independent vasodilation (glyceroltrinitrinate infusion) was not affected by allopurinol. In patients with normal UA levels, no effect of allopurinol coinfusion was observed.

Study 2: Placebo-Controlled Treatment Study

Fifteen male patients entered the trial. In 1 patient, an acute attack of gout occurred 3 days after the beginning of the trial. This patient was withdrawn from study medication and unblinded so as to direct further treatment. It was established that he was in the placebo phase. The remaining 14 patients completed the trial without adverse events; they remained clinically stable during the study, and no change in drug therapy was required. Compliance was 100% in all patients as assessed by counting of tablets returned. No significant differences were found in any of the baseline clinical characteristics between those patients allocated to allopurinol first (n=8) or placebo first (n=6); (Table 3). No significant changes in blood pressure, heart rate, weight, and creatinine (baseline 139±11 [range 93 to 194], treatment 136±10, placebo 133±10 μmol/L) were observed. Biochemical parameters (on liver function, kidney, and thyroid function) did not change significantly during the study (data not shown). UA was 558±21 μmol/L at baseline (range 455 to 743 μmol/L). Allopurinol reduced UA levels in all patients (P<0.0001, mean 217±16 μmol/L, range 122 to 330 μmol/L; Figure 1) compared with baseline.

There was no significant difference in blood flow results between baseline and placebo phase. After allopurinol treat-

TABLE 2. Allopurinol Infusion Study: Changes From Baseline of the Internal Diameter of the Radial Artery in Patients With Normal and Elevated Uric Acid Levels After Endothelium-Dependent and -Independent Stimulation and Coinfusion With Either Allopurinol or Saline

<table>
<thead>
<tr>
<th>Patients With Normal Uric Acid Levels (n=10)*</th>
<th>Patients With Elevated Uric Acid Levels (n=9)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anagostant-mediated endothelium-dependent vasodilation</td>
<td>Anagostant-mediated endothelium-dependent vasodilation</td>
</tr>
<tr>
<td>$Ach$ 7.5 μg/min</td>
<td>$Ach$ 30 μg/min</td>
</tr>
<tr>
<td>28±5</td>
<td>240±10</td>
</tr>
<tr>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>$Ach$ 30 μg/min</td>
<td>$Ach$ 30 μg/min</td>
</tr>
<tr>
<td>250±10</td>
<td>250±10</td>
</tr>
<tr>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Flow-dependent vasodilation</td>
<td>Flow-dependent vasodilation</td>
</tr>
<tr>
<td>390±15</td>
<td>370±20</td>
</tr>
<tr>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Endothelium-independent vasodilation</td>
<td>Endothelium-independent vasodilation</td>
</tr>
<tr>
<td>GTN 0.2 mg/min</td>
<td>GTN 0.2 mg/min</td>
</tr>
<tr>
<td>600±100</td>
<td>580±90</td>
</tr>
<tr>
<td>0.7</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$Ach$ indicates acetylcholine; GTN, glyceroltrinitrinate. Values are in micrometers.

*Normal uric acid levels, ≤440 μmol/L; elevated uric acid levels; >440 μmol/L.

TABLE 3. Placebo-Controlled Allopurinol Treatment Study: Patient Characteristics at Baseline for the 2 Crossover Study Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Allopurinol First (n=8)</th>
<th>Placebo First (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>68±2</td>
<td>69±3</td>
</tr>
<tr>
<td>Etiology, ischemic/dilative</td>
<td>5/3</td>
<td>6/0</td>
</tr>
<tr>
<td>NYHA class</td>
<td>2.4±0.3</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>Peak oxygen uptake, mL·min⁻¹·kg⁻¹</td>
<td>17.2±2.3</td>
<td>14.6±0.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.7±2.1</td>
<td>27.2±1.6</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>106±6</td>
<td>99±7</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>65±3</td>
<td>59±3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>63±2.6</td>
<td>65±7.0</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>130±16</td>
<td>150±14</td>
</tr>
<tr>
<td>Urate, μmol/L</td>
<td>535±22</td>
<td>588±37</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1. All parameters were not significantly different between the 2 groups: all P>0.2, except diastolic BP, P=0.14. χ² test for distribution of etiology, P=0.09.
ment, no significant change in resting arm or leg blood flow was recorded (Figure 2A). Compared with the placebo phase, however, allopurinol treatment improved stimulated blood flow. Allopurinol increased postischemic blood flow by 23% at the leg ($P=0.029$) and 24% at the arm vascular bed ($P=0.027$, Figure 2B). Flow-dependent flow was also significantly improved by allopurinol treatment (58%, $P=0.011$; Figure 2C).

Plasma allantoin levels at baseline were 26.1 ± 1.2 μmol/L. This is 75% higher than the mean value in healthy male subjects. After allopurinol treatment, allantoin levels were reduced by 20% to 20.8 ± 0.6 μmol/L ($P<0.001$). A significant carryover effect, however, was noted for allantoin. In the group treated with allopurinol in the first leg of the study, allantoin was also significantly reduced after the second leg of the study when patients had received only placebo (20.5 ± 0.5 versus 26.2 ± 1.7 μmol/L at baseline, $P<0.05$).

**Correlation Analysis**

To elucidate factors that predict the improvement of stimulated blood flow, simple linear regression analysis was performed for baseline parameters versus change of postischemic peak arm and leg blood flow and flow-dependent flow. None of the baseline characteristics significantly predicted improvement of blood flow. Also, no direct correlation could be found between treatment-induced change of allantoin and change of blood flow. The therapeutic effect of the reduction of UA from baseline, however, was directly related to the improvement of flow-dependent flow ($r=0.63$, $P<0.012$, Figure 3).

**Discussion**

In this study, we show that in patients with CHF and hyperuricemia, treatment with the XO inhibitor allopurinol improved endothelial function and increased stimulated postischemic and flow-dependent blood flow of the arm and leg vascular beds. Plasma allantoin levels decreased significantly after treatment with allopurinol. There is a direct relationship between the allopurinol-induced reduction of UA levels and improvement of flow-dependent flow.

The primary end point of the treatment study was change of peak leg blood flow, which increased by 23%. In daily life, CHF patients experience symptoms primarily during walking, i.e., during leg exercise. Accordingly, in clinical settings, exercise capacity is assessed primarily for the leg muscular system (through treadmill or bicycle exercise testing or 6-minute walking test). Consequently, the effects of allopurinol treatment on leg blood flow seem most relevant. In most studies, peripheral blood flow is assessed at the forearm only. We could show that the vasodilator capacity improved after allopurinol therapy in both arm and leg vascular beds to a similar degree. This suggests that the regulation of the vascular tone affected by allopurinol is similar in these 2 vascular regions.

XO is seen as a major source of increased oxygen free radical production, and this enzyme may contribute to impaired vasodilator capacity in CHF via upregulated oxidative stress. It should be noted that in humans, the tissue with the highest activity of XO, aside from the epithelium of the mammary gland, is the capillary endothelium and the endothelium of the small arteries. XO-generated radicals may reduce endothelium-derived vasoactive NO by formation of
ONOO⁻ (in itself a highly active oxygen radical). In accord with earlier reports,9,21 the allopurinol infusion study demonstrates a beneficial effect of intra-arterial allopurinol application on vasodilator capacity via endothelium-dependent mechanisms. The second study (using oral allopurinol treatment) demonstrates particular improvement in flow-dependent flow.

Measuring allantoin, a marker of oxygen free radical generation,14 we found that in CHF, allantoin is elevated compared with healthy subjects. There was no relation between allantoin and serum UA levels, which is in line with previous reports.22 The carryover effect seen for allantoin (but not for UA) suggests that allantoin is not merely a reflection of UA levels. From these results, it could be speculated that allopurinol treatment has prolonged effects on free radical accumulation in CHF.

We did not find a direct relationship between reduced plasma allantoin levels and changes in postschismic or flow-dependent blood flow. We found, however, that the treatment-induced reduction of UA correlated significantly with the improvement of flow-dependent flow. This might raise the possibility that UA itself may have an adverse effect on the regulation of peripheral vascular tone. We have previously reported that UA strongly and independently predicts impaired vasodilator capacity and increased leg vascular resistance in CHF patients.5 This does not contradict the findings from the infusion study, which suggest a direct endothelium-dependent mechanism. We would like to suggest that there might be a combination of effects of allopurinol treatment that act in synergy.

From the present study, we cannot prove a specific mechanism for the direct relation between change of UA and improvement of vasodilator capacity. Allopurinol does not affect autonomic tone.23 In CHF, UA levels relate to the degree of inflammatory activation.24 In this context, it was shown in a mouse model that UA infusion causes increased endotoxin-stimulated production of tumor necrosis factor-α.12 Tumor necrosis factor-α, in turn, can contribute to vascular damage. The role of UA should be viewed in the context of chronic tissue hypoxia that promotes a shift toward XO within the XO/xanthine dehydrogenase balance. Interestingly, inhibition of XO appears to exert direct myocardial effects in CHF. In animal models, allopurinol reduces myocardial oxygen consumption25 and improves systolic function,26 resulting in increased myocardial efficiency. This was recently confirmed in human CHF.27 Taken together, these data and our results suggest that treatment with allopurinol might be a promising novel treatment option having multiple effects toward improving both peripheral and central aspects of CHF.

One limitation of this study may be seen in the different methods of assessing vasodilator capacity in the 2 independent substudies presented. High-precision ultrasound, as used in the first study, is widely used for assessments of forearm endothelial dysfunction. For the assessment of the leg vascular bed, however, the ultrasound technique might be of limited value, because the femoral artery is often too big to accurately track changes in vessel diameter. For the assessment of stimulated limb vasodilator capacity, plethysmography is regarded as a highly reliable method.28 The finding that both techniques were in line with each other in demonstrating a similar effect of allopurinol to improve vasodilator capacity and endothelial function in hyperuricemic patients with CHF may harden the evidence provided by our 2 independent studies.

In summary, we have shown that in hyperuricemic patients with CHF, treatment with allopurinol leads to improved vasodilator capacity of the peripheral vascular bed. This is achieved by improved endothelial dysfunction but potentially also via a direct adverse effect of UA in the regulation of peripheral vascular tone. The combination of these mechanisms acting jointly may underlie the effect of allopurinol to improve peripheral vasodilator capacity. Whether this effect may translate into clinical benefit warrants further investigation.

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References


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