Allopurinol Improves Myocardial Efficiency in Patients With Idiopathic Dilated Cardiomyopathy

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Background—Dilated cardiomyopathy is characterized by an imbalance between left ventricular performance and myocardial energy consumption. Experimental models suggest that oxidative stress resulting from increased xanthine oxidase (XO) activity contributes to this imbalance. Accordingly, we hypothesized that XO inhibition with intracoronary allopurinol improves left ventricular efficiency in patients with idiopathic dilated cardiomyopathy.

Methods and Results—Patients (n=9; ejection fraction, 29±3%) were instrumented to assess myocardial oxygen consumption (MVO₂), peak rate of rise of left ventricular pressure (dP/dt max), stroke work (SW), and efficiency (dP/dt max/MVO₂ and SW/MVO₂) at baseline and after sequential infusions of intracoronary allopurinol (0.5, 1.0, and 1.5 mg/min, each for 15 minutes). Allopurinol caused a significant decrease in MVO₂ (peak effect, −16±5%; P<0.01; n=9) with no parallel decrease in dP/dt max or SW and no change in ventricular load. The net result was a substantial improvement in myocardial efficiency (peak effects: dP/dt max/MVO₂, 22±9%, n=9; SW/MVO₂, 40±17%, n=6; both P<0.05). These effects were apparent despite concomitant treatment with standard heart failure therapy, including ACE inhibitors and β-blockers. XO and its parent enzyme xanthine dehydrogenase were more abundant in failing explanted human myocardium on immunoblot.

Conclusions—These findings indicate that XO activity may contribute to abnormal energy metabolism in human cardiomyopathy. By reversing the energetic inefficiency of the failing heart, pharmacological XO inhibition represents a potential novel therapeutic strategy for the treatment of human heart failure. (Circulation. 2001;104:2407-2411.)

Key Words: heart failure ■ hemodynamics ■ free radicals

Heart failure is characterized by an imbalance between left ventricular performance and myocardial energy consumption, a phenomenon that is best described as mechanoenergetic uncoupling. Despite markedly impaired left ventricular work, the oxygen cost of contraction remains relatively unchanged, resulting in a decrease in the mechanical efficiency of contraction.¹ The clinical relevance of this phenomenon has been demonstrated by studies of inotropic agents, which worsen efficiency by increasing left ventricular work at the expense of disproportionate increases in myocardial oxygen consumption.² These unfavorable effects on energetics offer a possible explanation for the increased mortality associated with long-term inotropic therapy.³

The mechanisms responsible for mechanoeenergetic uncoupling are unknown, although experimental evidence suggests that reactive oxygen species play a major role. Markers of reactive oxygen species accumulate in the pericardial fluid⁴ and circulation⁵ of heart failure patients, indicating that heart failure is a state of oxidative stress.⁶ Although there are several potential sources of reactive oxygen species, increased levels of uric acid in the serum of heart failure patients suggest that xanthine oxidase (XO) activity contributes.⁷,⁸ XO is expressed as xanthine dehydrogenase (XDH), which is converted to XO via proteolysis or thiol oxidation.⁹ XO is a source of superoxide, and although there are conflicting reports in the literature, XO activity has been demonstrated in human myocardium.¹⁰ In animal models of heart failure, our group and others have shown that XO inhibition improves myocardial efficiency¹¹,¹² and enhances the contractile response of failing myocardium to dobutamine and to exercise.¹³ Whether XO inhibition has beneficial effects in human congestive heart failure is unknown. Thus, the purpose of this study was to examine the hemodynamic and energetic effects of XO inhibition in patients with idiopathic dilated cardiomyopathy. We tested the hypotheses that (1) short-term administration of intracoronary allopurinol improves left ventricular efficiency and (2) myocardial XO is upregulated in failing versus nonfailing human myocardium.

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Dr. Marbán holds equity in Paralex, Inc., a company that seeks to investigate the therapeutic potential of xanthine oxidase inhibitors for various indications. The present work was completed before Paralex was incorporated, and no financial support was received from Paralex.

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Methods

Patients
Nine patients (7 men, 2 women; mean ± SD age, 46 ± 10 years) with nonischemic dilated cardiomyopathy were recruited from the population of patients referred to the Johns Hopkins Cardiovascular Diagnostic Laboratory for elective coronary angiography. All participants had an ejection fraction < 40% by echocardiography, and none had coronary stenoses > 50%. Participants had a range of symptoms, including NYHA classes I (n = 1), II (n = 2), III (n = 5), and IV (n = 1). All participants were on standard medical therapy for congestive heart failure, including ACE inhibitors (n = 9), diuretics (n = 9), digoxin (n = 6), β-blockers (n = 5), and spironolactone (n = 2). The study protocol was approved by the Johns Hopkins Joint Committee on Clinical Investigation, and each patient provided written, informed consent.

Hemodynamic Measurements
Patients underwent routine diagnostic catheterization via the femoral approach. Nonionic contrast was used for coronary angiography and venography. A high-fidelity micromanometer-tipped conductance catheter (Millar) was placed in the left ventricular apex for simultaneous pressure and volume measurements. Technically reliable volume signals were obtained in 6 of the 9 participants and were calibrated to milliliters by use of ejection fraction and cardiac output. A Judkins L-4 catheter was placed in the ostium of the left main coronary artery, and an ultrasound-tipped wire (Flowire) was advanced through this catheter to measure left coronary flow velocity. A catheter was placed in the coronary sinus to allow repeated measurements of coronary mixed venous oxygen saturation. The ECG, aortic pressure, left ventricular pressure and volume, and coronary flow velocity were digitally recorded and analyzed with customized software. Each measurement represents the mean of ≥ 15 consecutive beats.

Drug Infusion
To target XO in the myocardium and to minimize possible peripheral effects, allopurinol was administered at a low dose directly into the left coronary artery. After instrumentation, intracoronary 5% dextrose in water was infused at 2 cm/min via the Judkins L-4 catheter for 10 minutes. Baseline hemodynamics, coronary flow velocity, and coronary sinus and arterial oxygen saturations were measured. Allopurinol prepared for parental use (Alloprim, Nabi) was then infused via the Judkins L-4 catheter at 0.5, 1.0, and 1.5 mg/min, each for 15 minutes. On the basis of an estimated coronary blood flow of 140 mL/min, these doses of allopurinol achieved steady-state concentrations of 26, 52, and 78 μmol/L in the coronary circulation.14 Hemodynamics, coronary flow velocity, and oxygen saturations were measured after each allopurinol infusion and were compared with baseline.

Data Analysis
Indexes of myocardial performance and ventricular load were derived from the steady-state pressure data in all 9 patients. A more detailed analysis was possible in the 6 patients for whom pressure-volume data were available. Preload was assessed as left ventricular end-diastolic pressure and volume. Afterload was assessed by measuring systemic vascular resistance and effective arterial elastance (Ea and systemic vascular resistance). Ees is a comprehensive measurement of total afterload on the left ventricle, encompassing systemic vascular resistance, aortic impedance, and the reflected wave properties of the vasculature.15 Myocardial contractility was assessed by determining the peak rate of rise of left ventricular pressure (dP/dt max). In addition, we measured the slope of the end-systolic pressure-volume relation (Ees), a load-independent measure of contractility, using a single-beat formula. This method, which derives a value for Ees, is one way to express contractility.16 Relaxation was assessed by use of the time constant, τ, of ventricular relaxation derived from left ventricular pressure recordings with a hybrid logistic method.17 Left main coronary diameter was measured with digitized angiograms. Left main coronary blood flow (Q cor) was calculated from the coronary flow velocity and left main diameter assuming laminar flow.18 Myocardial oxygen extraction (AV O2) was calculated as the difference between arterial and coronary sinus O2 saturations. Myocardial oxygen consumption (MV O2) was calculated from AV O2, Q cor, and blood hemoglobin concentration with the Fick equation. When necessary, MV O2 was converted into Joules per beat, assuming 20 J energy consumption per 1 mL O2 consumption.1 Left ventricular stroke work (SW) was calculated as the area enclosed by the pressure-volume loop and converted into Joules per beat. Myocardial efficiency was assessed as the ratio of myocardial contractility to myocardial oxygen consumption (dP/dt max/MVO2) and the ratio of SW to myocardial oxygen consumption (SW/MVO2).

Western Blots
Failing human myocardium was obtained at the time of cardiac transplantation from 5 patients with idiopathic dilated cardiomyopathy (2 men, 3 women; mean age, 51 ± 5 years). These patients had NYHA class III symptoms, which is similar to the average NYHA class (2.7) in the hemodynamic study. Three samples from unused donor hearts with normal ventricular function were used for comparison. Samples were lysed in buffer containing protease inhibitors (150 mM NaCl, 50 mM LiCl, Tris pH 7.6, 1 mM EDTA, 0.1% SDS, 1% sodium deoxycholate, 1% Triton X-100, 1 mM PMSF, 50 mM/mL sodium fluoride, 0.5 mM/mL sodium orthovanadate, 1 μg/mL leupeptin, and 2 μg/mL aprotinin). After centrifugation to remove cell fragments, the Bio-Rad assay was used to determine the total protein concentration in each sample. Equal amounts (150 μg) of each sample were resolved by SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 5% TBS milk, membranes were probed with a mouse monoclonal antibody directed against human xanthine oxidoreductase (Neomarker). Bands were detected by use of goat anti-mouse antibodies and a chemiluminescence system and were quantified by densitometry.

Statistical Analysis
All data are given as mean ± SEM. Hemodynamics and energetics were compared before and after intracoronary allopurinol by use of 2-tailed paired t tests. Dose-response curves were tested for statistical significance by use of a 1-way ANOVA for repeated measures and the Student-Newman-Keuls test. Densitometry results were compared by use of 2-tailed unpaired t tests. Statistical significance was defined as P < 0.05.

Results
The Table summarizes data at baseline and after allopurinol infusion. Study subjects exhibited the characteristic hemodynamic abnormalities of heart failure:15 reduced contractility (dP/dt max and Ees), prolonged ventricular relaxation (τ), increased preload (left ventricular end-diastolic pressure and volume), and increased afterload (Ea and systemic vascular resistance). Infusion of allopurinol decreased MV O2 from 17.2 ± 3.4 to 14.7 ± 3.4 mL O2/min (−15.8 ± 4.6%, P = 0.005). Despite the decrease in MV O2, there was no significant decrease in dP/dt max, Ees, or SW. The net result was a substantial increase in the efficiency of contraction. The ratio of dP/dt max to MV O2 increased from 222 ± 35 to 266 ± 40 mm Hg/J (22 ± 9%, P = 0.009). For the subset of patients for whom pressure-volume data were available, mechanical efficiency (SW/MVO2) improved from 34 ± 4% to 45 ± 5% (an increase of 38 ± 15%, P = 0.01). The decrease in MV O2 and improvement in efficiency were consistent among all patients studied (Figure 1A) and were not attributable to
The peak effects were a 16\% decrease in MVO\textsubscript{2}, a 22\% increase in dP/dt\textsubscript{max}/MVO\textsubscript{2}, and a 40\% increase in SW/MVO\textsubscript{2} (all P<0.05).

Data from animal models indicate that XO is upregulated in heart failure,\textsuperscript{12,19} but such a change has not been documented in human heart failure. To examine this possibility, we performed immunoblots of XO and its parent enzyme, XDH, in failing and nonfailing human myocardium. As indicated in Figure 3, bands corresponding to both XDH (145 kDa) and XO (125 and 85 kDa) are present in human myocardium. All bands are increased in cardiomyopathy, with a 60\% (P<0.05) overall increase in total XDH/XO protein content, indicating increased expression of the XDH gene and/or decreased XDH/XO protein degradation in cardiomyopathy.

Discussion

Our findings show that short-term administration of allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy by decreasing the oxygen cost of left ventricular contraction. Because allopurinol is a selective XO inhibitor, these results suggest that XO contributes to mechanoenergetic uncoupling in human heart failure. Furthermore, energetics improved after the selective infusion of allopurinol directly into the coronary circulation, implying that the relevant site of XO activity is the myocardium. These findings are supported by the increased XDH/XO protein abundance in failing compared with normal myocardium.

<table>
<thead>
<tr>
<th>Hemodynamic and Energetic Response to Intracoronary Allopurinol</th>
<th>Baseline</th>
<th>Allopurinol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure data (n=9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>80±5</td>
<td>81±4</td>
<td>0.27</td>
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<tr>
<td>SBP, mm Hg</td>
<td>132±8</td>
<td>136±8</td>
<td>0.15</td>
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<td>DBP, mm Hg</td>
<td>86±3</td>
<td>89±4</td>
<td>0.15</td>
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<tr>
<td>LVEDP, mm Hg</td>
<td>19±2</td>
<td>22±2</td>
<td>0.15</td>
</tr>
<tr>
<td>dP/dt\textsubscript{max}, mm Hg/s</td>
<td>1015±82</td>
<td>1010±81</td>
<td>0.74</td>
</tr>
<tr>
<td>τ, ms</td>
<td>62±2</td>
<td>65±4</td>
<td>0.31</td>
</tr>
<tr>
<td>Qcor, mL/min</td>
<td>142±23</td>
<td>122±23</td>
<td>0.005</td>
</tr>
<tr>
<td>AVO\textsubscript{2}, % saturation</td>
<td>66±3</td>
<td>66±3</td>
<td>0.62</td>
</tr>
<tr>
<td>MVO\textsubscript{2}, mL O\textsubscript{2}/min</td>
<td>17.2±3.4</td>
<td>14.7±3.4</td>
<td>0.019</td>
</tr>
<tr>
<td>MVO\textsubscript{2}, J/s</td>
<td>5.7±1.1</td>
<td>4.9±1.1</td>
<td>0.019</td>
</tr>
<tr>
<td>dP/dt\textsubscript{max}/MVO\textsubscript{2}, mm Hg/J</td>
<td>222±35</td>
<td>266±40</td>
<td>0.009</td>
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</table>

Pressure-volume data (n=6)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Allopurinol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDV, mL</td>
<td>248±28</td>
<td>242±28</td>
<td>0.29</td>
</tr>
<tr>
<td>LVEDV, mL</td>
<td>183±27</td>
<td>170±30</td>
<td>0.28</td>
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<tr>
<td>SV, mL</td>
<td>66±4</td>
<td>72±9</td>
<td>0.30</td>
</tr>
<tr>
<td>EF, %</td>
<td>29±3</td>
<td>32±6</td>
<td>0.30</td>
</tr>
<tr>
<td>SVR, dyne · s · cm\textsuperscript{-5}</td>
<td>1435±326</td>
<td>1547±404</td>
<td>0.39</td>
</tr>
<tr>
<td>E\textsubscript{s}, mm Hg/mL</td>
<td>2.0±0.3</td>
<td>2.2±0.4</td>
<td>0.35</td>
</tr>
<tr>
<td>E\textsubscript{es}, mm Hg/mL</td>
<td>1.8±0.3</td>
<td>1.7±0.2</td>
<td>0.56</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>5.0±0.4</td>
<td>5.4±0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>SW, J/beat</td>
<td>1.02±0.13</td>
<td>1.07±0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>MVO\textsubscript{2}, J/s</td>
<td>3.24±0.35</td>
<td>2.48±0.40</td>
<td>0.02</td>
</tr>
<tr>
<td>SW/MVO\textsubscript{2}, %</td>
<td>34±4</td>
<td>45±5</td>
<td>0.01</td>
</tr>
</tbody>
</table>

HR indicates heart rate; SBP, aortic systolic pressure; DBP, aortic diastolic pressure; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; SV, stroke volume; EF, ejection fraction; SVR, systemic vascular resistance; E\textsubscript{s}, effective arterial elastance; E\textsubscript{es}, end-systolic elastance; and CO, cardiac output. Probability values were computed from paired t-tests.

As summarized in Figure 1B, the improvement in energetics was due mainly to a decrease in Qcor, with minimal change in oxygen extraction. To assess whether this was a sign of epicardial coronary vasconstriction, we measured coronary artery diameter before and after allopurinol in 6 of the 9 patients. There was no significant change in proximal left main coronary artery diameter (5.11±0.45 and 4.91±0.32 mm before and after allopurinol respectively; -2±5\%; P=NS). Figure 2 displays the change in energetics that accompanied each dose of intracoronary allopurinol. Improved efficiency was nearly maximum at the lowest infusion rate of 0.5 mg/min (27 \textmu mol/L) and peaked at 1.0 mg/min (52 \textmu mol/L). The lack of a linear dose-response relationship may be a result of our relatively high initial infusion rate, which is similar to the expected plasma concentration of a 600-mg oral dose of allopurinol (20 \textmu mol/L). The peak effects were a 16±5\% decrease in MVO\textsubscript{2}, a 22±9\% increase in dP/dt\textsubscript{max}/MVO\textsubscript{2}, and a 40±17\% increase in SW/MVO\textsubscript{2} (all P<0.05).

Data from animal models indicate that XO is upregulated in heart failure,\textsuperscript{12,19} but such a change has not been documented in human heart failure. To examine this possibility, we performed immunoblots of XO and its parent enzyme, XDH, in failing and nonfailing human myocardium. As indicated in Figure 3, bands corresponding to both XDH (145 kDa) and XO (125 and 85 kDa) are present in human myocardium. All bands are increased in cardiomyopathy, with a 60\% (P<0.05) overall increase in total XDH/XO protein content, indicating increased expression of the XDH gene and/or decreased XDH/XO protein degradation in cardiomyopathy.
Although oxidative stress \(^6\) and abnormal energy metabolism \(^1\) are established features of heart failure, our data suggest that they are linked pathogenetically. Free radical production by XO may be an important cause of impaired myocardial energy utilization, and this abnormality may be correctable pharmacologically. Thus, XO inhibition may offer a novel therapeutic strategy for the treatment of congestive heart failure.

Numerous investigations of XO in cardiovascular disease have focused on the vascular effects of XO and its role in ischemia/reperfusion injury.\(^{20,21}\) We observed improved energy metabolism after XO inhibition in patients with no evidence of myocardial ischemia or vascular disease. Because the primary change after allopurinol was a decrease in Q\(_{cor}\) without any significant change in aortic or left ventricular diastolic pressure, an increase in coronary resistance can be inferred. This was not caused by decreased epicardial coronary diameter, which remained unchanged, but may have been due to small vessel vasoconstriction after allopurinol. In contrast, other antioxidants such as vitamin C have been shown to increase flow-dependent dilation in peripheral conduit arteries in heart failure,\(^{22}\) and cross-sectional studies have demonstrated an inverse correlation between serum uric levels and peripheral endothelial function.\(^{23,24}\) These studies suggest that XO inhibition should decrease vascular resistance. The increased coronary resistance in our study may reflect differences between coronary and peripheral vascular beds in heart failure. For example, although heart failure is characterized by peripheral vasoconstriction and decreased systemic perfusion, coronary blood flow is actually increased in idiopathic dilated cardiomyopathy.\(^{25}\) However, our ability to draw firm conclusions is limited because we did not perform a detailed evaluation of coronary endothelial function (such as with adenosine or acetylcholine challenge) after the administration of allopurinol. Further investigation is required to address these questions.

If an increase in coronary vascular tone were the only effect of allopurinol, one would expect a small increase in AVO\(_2\) to preserve net oxygen delivery to the myocardium. In fact, we observed no change in AVO\(_2\) and a decrease in myocardial oxygen consumption. That is, in addition to a vascular effect, there was a change in myocardial metabolism after allopurinol so that less oxygen was consumed overall by the heart without any detrimental effects on cardiac function. These results are consistent with the ability of XO inhibitors to improve the SW/MVO\(_2\) ratio in canine pacing-induced heart failure and to act as calcium sensitizers in vitro.\(^{11,12}\) Unlike our results, the decrease in MVO\(_2\) observed in these model systems was accompanied by an increase in contractility. The absence of an inotropic effect in our study may reflect biological differences between these model systems and human disease. Alternatively, the response to allopurinol may have been blunted in our study by long-term therapy with vasodilators and neurohormonal-blocking agents. In either case, our results are notable in that contractility did not decrease despite the fall in energy consumption. The response to allopurinol despite concomitant heart failure therapy suggests that allopurinol may, in principle, confer added clinical benefit.

The mechanism by which XO inhibition improves myocardial efficiency has been shown in animal models to be due to enhanced myofilament responsiveness to activator calcium.\(^{13}\) However, the signaling mechanisms that underlie this response remain to be elucidated. One possibility is that superoxide produced by XO interferes with intracellular signals that are important regulators of energy metabolism. For example, nitric oxide regulates enzymes involved in ATP production,\(^{26}\) high-energy phosphate storage via creatine phosphokinase,\(^{27}\) and energy consumption by cardiac myocyte calcium cycling.\(^{28,29}\) Superoxide can react rapidly with
nitric oxide and may disrupt these signaling pathways, resulting in a dysregulation of energy metabolism and a decrease in efficiency. The possibility that our observations are due to a novel property of allopurinol independent of XO should be considered. However, the observation that another specific XO inhibitor, oxypurinol, has similar effects on cardiac muscle experimentally supports XO inhibition as the relevant mechanism.11

The most important limitations of this paired study are its small sample size and the lack of a parallel control group. Because accurate assessment of ventricular energetics requires an invasive protocol with extensive instrumentation, we aimed to prove a pathophysiological principle using the minimum number of patients. Accordingly, we used a paired study design in which each patient served as his or her own control. In the absence of a comparison group with normal left ventricular function, we cannot determine whether the changes in energetics we observed are specific to dilated cardiomyopathy or are present with normal cardiac function as well. In fact, the recent demonstration that vitamin C improves the contractile response to dobutamine in patients with normal ventricular function suggests that oxidative “stress” affects cardiac function to some degree even in the normal heart.30 One would expect this mechanism to be accentuated in heart failure, in which oxidative stress is increased. We measured only the short-term effects of allopurinol, and their translation into meaningful clinical improvements requires future clinical trials.

In summary, this study has shown that allopurinol reverses, at least in part, the mechanoenergetic uncoupling characteristic of human heart failure. Because XO inhibitors have a well-established safety profile and are used widely for the treatment of hyperuricemia and gout, the potential clinical benefits of these agents are worth further exploration.

Acknowledgments

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References

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