Allopurinol Attenuates Left Ventricular Remodeling and Dysfunction After Experimental Myocardial Infarction
A New Action for an Old Drug?

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Background—Accumulating evidence suggests a critical role for increased reactive oxygen species (ROS) production in left ventricular (LV) remodeling and dysfunction after myocardial infarction (MI). Increased expression of xanthine oxidase (XO), a major source of ROS, has recently been demonstrated in experimental and clinical heart failure; however, a potential role for LV remodeling processes remains unclear. We therefore studied the effect of long-term treatment with allopurinol, a potent XO inhibitor, on myocardial ROS production and LV remodeling and dysfunction after MI.

Methods and Results—Mice with extensive anterior MI (n = 105) were randomized to treatment with either vehicle or allopurinol (20 mg · kg⁻¹ · d⁻¹ by gavage) for 4 weeks starting on day 1 after surgery. Infarct size was similar among the groups. XO expression and activity were markedly increased in the remote myocardium of mice after MI, as determined by electron spin resonance spectroscopy. Myocardial ROS production was increased after MI but markedly reduced after allopurinol treatment. Importantly, allopurinol treatment substantially attenuated LV cavity dilatation and dysfunction after MI, as assessed by echocardiography, and markedly reduced myocardial hypertrophy and interstitial fibrosis.

Conclusion—The present study reveals a novel beneficial effect of treatment with allopurinol, ie, a marked attenuation of LV remodeling processes and dysfunction after experimental MI. Allopurinol treatment therefore represents a potential novel strategy to prevent LV remodeling and dysfunction after MI. (Circulation. 2004;110:2175-2179.)

Key Words: remodeling ■ free radicals ■ heart failure

Left ventricular (LV) remodeling processes after myocardial infarction (MI) remain a major challenge and contribute to the development and progression of heart failure.¹ Accumulating evidence suggests that increased myocardial reactive oxygen species (ROS) production plays a critical role in cellular signaling pathways leading to hypertrophy, dilatation, and dysfunction of the LV after MI.² Of note, increased expression and activity of xanthine oxidase (XO), a potent enzymatic source of ROS, have recently been demonstrated in experimental and clinical heart failure.³⁵ In vitro, direct exposure of cardiomyocytes to XO-derived oxidants has been shown to promote cardiomyocyte hypertrophy and dysfunction.⁶⁷

Given these observations, we sought to assess the effect of treatment with allopurinol, a potent inhibitor of XO, on myocardial ROS production, LV remodeling, and LV function after experimental MI in vivo.

Methods

Animals, MI, and Experimental Protocol
In male C57BL/6 mice, aged 14 to 16 weeks, MI was induced by permanent ligation of the left anterior descending coronary artery as described previously.⁸ Only mice with extensive MI (>30%) were included in the protocol (n = 105). On day 1 after MI, mice were randomized (1:2) to treatment with allopurinol (20 mg/kg daily by gavage, n = 35) or vehicle (n = 70) for 30 days. This dose of allopurinol provides effective inhibition of XO in mice.⁹ Thirteen sham-operated mice served as controls. Systolic blood pressure and heart rate were measured by a computerized tail-cuff system (Visitech Systems) as described previously.¹⁰ All animal experiments were approved by the local committee on animal research.

Immunoblot Analysis of XO
Protein extracts (20 μg) from the remote LV myocardium (ie, the free LV wall) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to membranes, and immunoblottedted with a monoclonal anti-XO antibody (NeoMarkers). Protein was visualized with an enhanced chemiluminescence detection system.

Measurement of XO Activity by ESR Spectroscopy
Activity of XO was determined in remote myocardium (10 μg protein) by electron spin resonance (ESR) spectroscopy as described previously³ by using the spin trap 1-hydroxy-3-carboxypyrrolidine and a MiniScope ESR spectrometer (Magnettech). The intensity of ESR spectra was quantified after
subtraction of the ESR signal of samples without xanthine (obtained for each sample).

**Measurement of Myocardial Superoxide Production**

Superoxide production was measured in remote myocardium by using the superoxide dismutase (SOD)–inhibitable cytochrome c reduction assay as described previously. Additional studies were performed with dihydroethidium fluorescence staining for in situ detection of superoxide production as described previously.

Matched pairs of myocardial samples from mice after MI and sham-operated mice were processed simultaneously, and tissue sections were visualized by confocal microscopy with identical acquisition parameters.

**Echocardiographic Measurements**

Echocardiography studies were performed under light anesthesia (100 mg/kg ketamine, 1.25 mg/kg xylazine, and 0.6 mg/kg atropine IP) and spontaneous respiration with a commercially available ultrasound system (ATL5000 CV) with a linear 15-MHz, high-frequency transducer as described previously. We have recently reported high reproducibility with this approach in mice. The observer was blinded to the experimental group assignment.

**Histomorphometric Analysis**

After fixation, LV tissue slices were embedded in paraffin, cut into 6-μm sections, and stained with collagen-specific Sirius red F3BA as described previously. Interstitial collagen volume fraction was quantified by polarized light microscopy of picrosirius red–stained LV tissue sections (Axiovert 100, Zeiss; original magnification ×400). Tissue morphometry was performed in a blinded fashion with the Quantimet 500MC digital image analyzer. Mean cardiomyocyte cross-sectional area and infarct size were determined in hematoxylin-eosin–stained sections with a digital image analyzer as described previously.

**Statistical Analysis**

All data are expressed as mean±SEM. To compare data between groups, ANOVA was used. A value of $P<0.05$ was considered statistically significant.

**Results**

Myocardial XO Protein Expression and Activity

XO protein expression was increased by $>1.4$-fold in the remote LV myocardium (free LV wall) after MI compared

Figure 1. A, XO/xanthine dehydrogenase immunoblot analysis. Bands corresponding to both XO (130 and 90 kDa) and xanthine dehydrogenase (150 kDa) are present. Densitometry analysis of XO/xanthine dehydrogenase expression revealed 60% increase in remote LV myocardium 30 days after MI compared with sham-operated mice. n=10 each (mean±SEM). B, XO activity as determined by ESR spectroscopy. Representative myocardial XO-dependent ESR spectra of CP from sham-treated mice and post-MI mice are shown. Allo indicates allopurinol. C, XO activity in myocardium of sham-treated and post-MI mice. n=10 each (mean±SEM). D, Superoxide production in myocardium of sham-treated post-MI mice as assessed by SOD-inhibitable cytochrome c reduction. n=8 to 17 (mean±SEM). E, In situ detection of myocardial superoxide production with dihydroethidium in sham-treated and post-MI mice. Data are representative of 3 separate experiments. All other abbreviations are as defined in text.
Myocardial Superoxide Production

Superoxide production was significantly increased in the remote LV myocardium (free LV wall) after MI as evaluated by both SOD-inhibitable cytochrome c reduction and in situ detection of superoxide production by dihydroethidium staining (Figure 1D and 1E). Both approaches revealed a significant reduction of superoxide production in the remote LV myocardium after MI treatment with allopurinol (Figure 1D and 1E). Representative ESR scans of myocardial XO activity are shown in Figure 1B.

LV Structure and Function

LV cavity dilation as assessed by end-diastolic diameter was substantially reduced after allopurinol treatment after MI compared with vehicle-treated mice (Table and Figure 2A). Furthermore, LV function as assessed by LV ejection fraction and LV fractional shortening was markedly impaired in mice after MI (Figure 2C). Treatment with allopurinol resulted in a substantial improvement of both LV ejection fraction and LV fractional shortening (Table and Figure 2C).

Myocardial Hypertrophy

Myocardial hypertrophy as assessed by both cardiomyocyte cross-sectional area (313.4 ± 64.1 μm^2, P < 0.05; Figure 2D) and LV weight/body weight ratio was markedly reduced after treatment with allopurinol in mice after MI (Table).

Myocardial Fibrosis

Treatment with allopurinol caused a significant reduction of the interstitial collagen volume fraction in mice after MI (Figure 2E).

Blood Pressure, Heart Rate, Infarct Size, and Mortality

The blood pressures and heart rates were analyzed at different time points after MI and are shown in the Table. Infarct size did not differ between the groups (Table). There was no significant difference with respect to survival 30 days after MI: 36 mice (51%) in the vehicle group died and 18 (51%) in the allopurinol-treated group died after MI.

Discussion

Our findings demonstrate that treatment with allopurinol has a marked beneficial effect on LV remodeling processes, ie, LV dilation, hypertrophy, and interstitial fibro-

### Echocardiographic Parameters, Histomorphometric Analysis, Blood Pressure, and Heart Rate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>MI</th>
<th>MI + Allopurinol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LV fractional shortening, % (n=7–15)</td>
<td>37.1 ± 1.1</td>
<td>9.9 ± 2.2*</td>
<td>19.5 ± 2.3§</td>
</tr>
<tr>
<td>LV ejection fraction, % (n=7–15)</td>
<td>55.9 ± 3.1</td>
<td>19.6 ± 4.5*</td>
<td>33.4 ± 4.7§</td>
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<tr>
<td>LV end-diastolic diameter, mm (n=7–15)</td>
<td>3.7 ± 0.1</td>
<td>6.0 ± 0.2*</td>
<td>4.9 ± 0.2§</td>
</tr>
<tr>
<td>LV-PWd, mm</td>
<td>0.5 ± 0.04</td>
<td>0.6 ± 0.03†</td>
<td>0.5 ± 0.02§</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>0.6 ± 0.03</td>
<td>0.6 ± 0.03</td>
<td>0.5 ± 0.03</td>
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<tr>
<td><strong>Histomorphometric analysis</strong></td>
<td></td>
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<tr>
<td>Mean infarct size, %</td>
<td>N/A</td>
<td>41.9 ± 5.5</td>
<td>46.0 ± 3.8</td>
</tr>
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<td>Myocyte CSA, μm²</td>
<td>259 ± 12</td>
<td>543.1 ± 64.1*</td>
<td>313.4 ± 8.7†</td>
</tr>
<tr>
<td>LV weight/body weight, mg/g</td>
<td>3.6 ± 0.1</td>
<td>5.2 ± 0.2*</td>
<td>4.4 ± 0.2§</td>
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<td><strong>Blood pressure, heart rate</strong></td>
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<td>Systolic blood pressure, mm Hg (n=12–22)</td>
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<tr>
<td>Preop</td>
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<td>115 ± 4</td>
<td>111 ± 3</td>
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<tr>
<td>Postop, day 1</td>
<td>107 ± 2</td>
<td>103 ± 3</td>
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<tr>
<td>Postop, day 14</td>
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<td>110 ± 4</td>
<td>104 ± 4</td>
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<tr>
<td>Heart rate, bpm</td>
<td>608 ± 19</td>
<td>624 ± 18</td>
<td>673 ± 15</td>
</tr>
</tbody>
</table>

LV-PWd indicates LV posterior wall end-diastolic diameter; IVSd, interventricular septum end-diastolic diameter; CSA, cross-sectional area; and N/A, not applicable. All other abbreviations are as defined in text.

*P<0.01 vs sham; †P<0.05 vs sham; ‡P<0.01 vs MI; §P<0.05 vs MI.
sis after experimental MI. Furthermore, treatment with allopurinol resulted in substantially improved LV function after MI. These beneficial effects of allopurinol were associated with a profound inhibition of XO in remote LV myocardium as revealed by ESR spectroscopy analysis and markedly reduced myocardial ROS production, suggesting that allopurinol exerts beneficial effects, at least in part, by inhibiting XO and reducing myocardial oxidant stress.

Recent in vitro studies have shown that exposure of cardiomyocytes to XO-derived oxidants stimulates a hypertrophic response, suggesting that allopurinol may, at least in part, reduce cardiomyocyte hypertrophy by inhibiting XO-mediated ROS production. Furthermore, exposure of cardiomyocytes to XO-derived oxidants in vitro induces rapid contractile dysfunction, which may partly explain the beneficial effect of allopurinol treatment on LV function after MI as observed in the present study. In addition, a calcium-sensitizing effect of allopurinol on cardiac myofilaments has recently been observed that may contribute to its beneficial effects on LV function. Notably, short-term allopurinol administration exerted a positive inotropic effect without increasing myocardial oxygen consumption, indicating improved myocardial efficiency after allopurinol.

The beneficial effects of allopurinol treatment observed in the present study were not attributable to an MI size-sparing effect, because infarct sizes were similar between the groups. Whether there is an infarct size-sparing effect when allopurinol treatment is started earlier needs to be addressed by future studies.

Myocardial XO expression has been shown to be increased in patients with chronic heart failure (CHF). Moreover, we have recently observed by using ESR spectroscopy an increased endothelium-bound XO activity related to oxidant stress in patients with CHF, compatible with the concept of XO activation in human heart failure. Notably, in all these studies, patients were on angiotensin-converting enzyme inhibitor treatment, suggesting that XO is not sufficiently suppressed by this therapy. Moreover, treatment with allopurinol reduces circulating markers of oxidative stress in patients with CHF, and elevated serum levels of uric acid, the product of XO/xanthine dehydrogenase, have been identified as an independent prognostic predictor in patients with CHF. The novel findings of the
The present study may therefore importantly contribute to understanding the potential beneficial effects of allopurinol treatment in heart failure. Of note, clinical trials are ongoing to analyze the effect of treatment with oxypurinol, the major metabolite of allopurinol, on morbidity and mortality in patients with CHF (ie, OPT-CHF17).

**Study Limitations**

The present study was not designed to determine whether peripheral effects of allopurinol treatment may have contributed to improved LV remodeling and function after MI. Analysis of resting hemodynamics after MI did not reveal a significant reduction of blood pressure or heart rate after allopurinol treatment. Recent studies, however, have demonstrated an improved endothelium-dependent vasodilation in conductance and resistance vessels after allopurinol treatment in CHF.15,18 It is therefore conceivable that there may be an afterload reduction in particular during exercise that needs to be addressed by future studies. In the present study, there was no significant improvement of survival after allopurinol treatment within 30 days after MI. Whether allopurinol treatment, in addition to improving LV function and remodeling after MI, can reduce mortality needs to be addressed by future studies with a long-term follow-up. XO and oxygen radical production were analyzed in remote LV myocardium in the present study, where a marked cardiomyocyte hypertrophy was observed. This does not exclude, however, that allopurinol may also have exerted effects in infarcted parts of the LV that could have contributed to attenuation of LV dilation.

In summary, the present study demonstrates a novel beneficial effect of allopurinol, ie, a marked attenuation of LV remodeling processes after experimental MI, and an improved LV function likely mediated, at least in part, by reduced myocardial XO activity and ROS production.

**References**


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