Impact of elevated uric acid on ventricular remodeling in infarcted rats with experimental hyperuricemia

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Hyperuricemia is associated with cardiovascular disease, but it is usually considered a marker rather than a risk factor. Previous studies using uric acid-lowering drugs in normouricemic animals are not suitable to answer the effect of hyperuricemia on ventricular remodeling after myocardial infarction. The purpose of this study was to determine whether hyperuricemia adversely affects ventricular remodeling in infarcted rats with elevated uric acid. Male Wistar rats aged 8 wk were randomly assigned into either vehicle, oxonic acid, oxonic acid + allopurinol, oxonic acid + benz bromarone, oxonic acid + ABT-627, or oxonic acid + tempol for 4 wk starting 24 h after ligation. Postinfarction was associated with increased oxidant production, as measured by myocardial superoxide, isoprostane, xanthine oxidase activity, and dihydrothidium staining. Compared with normouricemic infarcted rats, hyperuricemic infarcted rats had a significant increase of superoxide production (1.7×) and endothelin-1 protein (1.2×) and mRNA (1.4×) expression, which was associated with increased left ventricular dysfunction and enhanced myocardial hypertrophy and fibrosis. These changes were all prevented by treatment with allopurinol. For similar levels of urate lowering, the uricosuric agent benz bromarone had no effect on ventricular remodeling. In spite of equivalent hyperuricemia, the ability of both ABT-627 and tempol to attenuate ventricular remodeling suggested involvement of endothelin-1 and redox pathways. Hyperuricemia is associated with unfavorable ventricular remodeling probably through a superoxide and endothelin-1-dependent pathway. Uric acid lowering without inhibition of superoxide and endothelin-1 may not have an effect on remodeling. Chronic administration of allopurinol, ABT-627, and tempol is associated with attenuated ventricular remodeling.

endothelin-1; myocardial infarction; superoxide; ventricular remodeling

Epidemiologic studies have shown that elevated uric acid is associated with risk of cardiovascular disease death in apparently healthy middle-aged men from the general population (25). Gouty arthritis is associated with an excess risk of acute myocardial infarction (MI), and this is not explained by its well-known links with renal function, metabolic syndrome, diuretic use, and traditional cardiovascular risk factors (18). High uric acid concentrations on admission were strongly associated with adverse clinical outcome in patients with MI (16). Despite the association of uric acid with prognosis, controversy exists as to whether uric acid has an etiologic role.

Differences in measurement of and adjustment for potential confounding factors in clinical studies could have contributed to the discrepancies. Thus further animal studies with homogeneous baseline are needed to investigate the pathophysiological mechanisms by which high serum uric acid is associated with prognosis after MI. To date, no experimental studies involving elevated uric acid models have shown the effect of hyperuricemia on ventricular remodeling. Much of the recent work has relied on lowering uric acid after the induction of MI in animals with normouricemia. Extending these results to hyperuricemic animals might underestimate the effect of uric acid on ventricular remodeling. Furthermore, in clinical trials, elevated uric acid at baseline is far more present in the general population than was anticipated before, making our proposal notable from a translational perspective.

Cardiac remodeling is an unfavorable evolution associated with myocardial hypertrophy, fibrosis, and ventricular dysfunction after MI (43). Cardiac remodeling is a complex process involving numerous signaling pathways. We and others (15, 20) have shown that the production of reactive oxygen species (ROS) is increased in post-MI remodeling, and pharmacological interventions to scavenge ROS can ameliorate this disease process. Growing evidence suggests an important role for increased oxidative stress in adverse left ventricular (LV) remodeling after MI (40, 52). Xanthine oxidase (XO) expression and activity, as determined by electron spin resonance spectroscopy, were found to be markedly increased in the remote myocardium (>2 mm outside the infarct) of mice after MI (7). Blocking XO-generated oxygen radical accumulation has emerged as an intriguing new treatment option for preventing oxygen radical accumulation and its adverse effects. Endothelin (ET)-1, a potent growth-promoting peptide derived from endothelial cells, is also produced by cardiac myocytes (26). ET-1 acts as a key autocrine/paracrine mediator to trigger the hypertrophic signaling pathways by activation of extracellular signal-regulated kinase in myocardium (51). The ET system is a promising candidate for cardiac remodeling after MI because ET-1 receptor blockade attenuates ventricular remodeling (19). Recently, ROS has been shown to increase ET-1 expression via the activation of extracellular signal-regulated kinases in cardiac fibroblasts (5). The present study tested the hypotheses that hyperuricemia induced by oxonic acid stimulates myocardial superoxide production, resulting in enhanced ET-1-induced ventricular remodeling in infarcted rats. Furthermore, we determined whether the treatment of hyperuricemic rats with allopurinol and benz bromarone, uric acid-lowering agents, could attenuate this process after MI. To further confirm the role of ROS and ET-1 in the ventricular remodeling in hyperuricemic rats, infarcted rats with the SOD...
mimetic tempol and the ETα receptor blocker ABT-627 were also assessed.

**METHODS**

**Animals.** Male Wistar rats aged 8 wk (300–350 g) were subjected to ligation of the anterior descending artery as previously described (4) resulting in infarction of the LV free wall. Rats were randomly assigned into either vehicle (saline), oxonic acid (2%; Sigma, St. Louis, MO), oxonic acid + allopurinol (50 mg·kg body wt⁻¹·day⁻¹; Sigma), oxonic acid + benz bromarone (10 mg·kg body wt⁻¹·day⁻¹), oxonic acid + ABT-627 (5 mg·kg body wt⁻¹·day⁻¹); a selective ETα receptor antagonist; Abbott Park, IL), or oxonic acid + tempol (15 mg·kg body wt⁻¹·day⁻¹), a stable membrane-permeable SOD mimetic; Sigma). The doses of oxonic acid and tempol have been shown to effectively modulate biological activities (33, 34). The doses of allopurinol and benz bromarone used in this study have been shown to effectively decrease uric acid levels without significantly changing blood pressure (28, 47).

The drugs were started 24 h after infarction, at a time when they could produce maximum benefits (45). The study duration was designed to be 4 wk because the majority of the myocardial remodeling process in the rat (70–80%) is complete within 3 wk (3). The drugs were administered by daily oral gavage. Sham-operated rats served as controls to exclude the possibility that the drugs themselves directly modulated ventricular remodeling. In each treated group, drugs were withdrawn about 24 h before the end of the experiments to eliminate their pharmacological actions. The animal experiment was approved by and conducted in accordance with local institutional guidelines for the care and use of laboratory animals at the Chi-Mei Medical Center and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

**Echocardiogram.** At 28 days after operation, rats were lightly anesthetized with intraperitoneal injection of ketamine HCl (25 mg/kg). Echocardiographic measurements were done with a HP Sonos 5500 system with a 15–6L (6–15 MHz; SONOS 5500; Agilent Technologies, Palo Alto, CA) probe as previously described (21). M-mode tracing of the LV was obtained from the parasternal long-axis view to measure LV end-diastolic diameter dimension and LV end-systolic diameter dimension, and fractional shortening (%) was calculated according to the American Society for Echocardiology (36). Measurements represented the mean of at least five consecutive cardiac cycles. Analyses were performed by an experienced observer blinded to the treatment groups to which the animals were allocated. After this, the hearts quickly underwent hemodynamic measurement after systemic heparinization.

**Hemodynamics and infarct size measurements.** Hemodynamic parameters were measured in anesthetized rats with an additional intra-peritoneal dose of ketamine (90 mg/kg) at the end of the echocardiogram. A polyethylene Millar catheter was inserted into the LV and connected to a transducer (Model SPR-407; Miller Instruments, Houston, TX) to measure LV systolic and diastolic pressure as the mean of measurements of five consecutive pressure cycles as previously described (21). The maximal rate of LV pressure rise (+dP/dt) and decrease (−dP/dt) was measured. After the arterial pressure measurement, the atria and the right ventricle were trimmed off, and the LV was rinsed in cold physiological saline, weighed, and immediately frozen in liquid nitrogen after obtaining a coronal section of the LV for infarct size estimation. A section, taken from the equator of the LV, was fixed in 10% formalin and embedded in paraffin for determination of infarct size. Each section was stained with hematoxylin and eosin and trichrome. The infarct size was determined as previously described (21). With respect to clinical importance, only rats with large infarct (>30%) were selected for analysis.

**Morphometric determination of myocyte size and fibrosis.** Because ventricular remodeling after infarction is a combination of reactive fibrosis and myocyte hypertrophy, we measured cardiomyocyte sizes in addition to myocardial weight to avoid the confounding influence of nonmyocytes on cardiac hypertrophy. LV sections from the remote zone (>2 mm outside the infarct) were stained with hematoxylin and eosin. For consistency of results, myocytes positioned perpendicularly to the plane of the section with a visible nucleus and cell membrane clearly outlined and unbroken were then selected for the cross-sectional area measurements (23). This area was determined by manually tracing the cell contour on a digitized image acquired on the image-analysis system at a magnification of 400 using computerized planimetry (Image Pro Plus) as previously described (22). A total of 100 myocytes were selected in the LV of each heart and analyzed by an observer blinded to the experimental treatment.

Additionally, heart sections were stained with Sirius red stain to distinguish areas of connective tissue as previously described (23). The percentage of red staining, indicative of fibrosis, was measured (10 fields randomly selected on each section). The value was expressed as the ratio of Sirius red-stained fibrosis area to total infarct area. All sections were evaluated under blinded conditions with prior knowledge as to which section belonged to which rat.

**In situ detection of superoxide.** For evaluating myocardial intracellular superoxide production using in situ dihydroethidium (DHE; Invitrogen Molecular Probes, Eugene, OR) fluorescence, optimal cutting temperature media-embedded tissues were sectioned (10 μm) at −20°C. After being fixed, tissues were incubated with DHE in PBS (10 μM) in a dark, humidified container at room temperature for 30 min. Generation of superoxide radicals by tissue was demonstrated by a red fluorescence, which was detected through a 580-nm long pass filter, using a digital camera mounted on an Olympus fluorescent microscope. The density of the images was reported as arbitrary units per millimeter square field (50).

**Western blot analysis of ET-1.** Samples obtained from the remote zone underwent Western blot as described previously (21). Primary antibody was anti-ET-1 (Immuno-Biological Lab, Gunma, Japan). Experiments were replicated three times, and results are expressed as the mean value.

**Real-time RT-PCR.** Real-time quantitative reverse RT-PCR was performed from samples obtained from the remote zone with the TaqMan system (Prism 7700 Sequence Detection System; PE Biosystems). For ET-1, the primers were 5′-TCCTGTGTTTGTGGCTTTC-3′ and 5′-CAAGGATCGCTTAGACCTAGAAGG. For cyclophilin, the primers were 5′-ATGGTCACAACCCACCGTGTCTTCG-3′ and 5′-CGTGTGAAGTCACCACA-3′. Cyclin D1 mRNA was chosen as the internal standard because it is expressed at a relatively constant level in virtually all tissues. For quantification, ET-1 expression was normalized to the expressed housekeeping gene cyclophilin. Reaction conditions were programmed on a computer linked to the detector for 40 cycles of the amplification step.

**Laboratory measurements.** Blood samples from the aorta were assayed at the end of the study. Blood samples were immediately centrifuged at 3,000 g for 10 min, and the serum was stored at −70°C until further analysis. The serum uric acid concentration was measured by the colorimetric uricase method using a commercial kit (King Diagnostics).

**Superoxide production by myocardium from the remote zone was measured using lucigenin (5 μM bis-N-methylacridinium nitrate; Sigma) enhanced chemiluminescence as previously described (22). The specific chemiluminescence signal was calculated after subtraction of background activity and expressed as counts per minute per milligram weight.**

Myocardial tissue free 15-F2t-isoprostane, a reliable index for in vivo oxidative stress-induced lipid peroxidation (27), was measured by using an EIA kit (Cayman Chemical; Ann Arbor, MI). Homogenized heart tissue (in PBS) from the remote zone was purified using Affinity Sorbent/Column (Cayman Chemical) in the presence of 0.01% butylated hydroxytoluene and then processed for analysis of 15-F2t-isoprostane as previously described (46). The values of heart
rats were tested by ANOVA. In case of a significant effect, the statistical analysis was performed using the SPSS statistical package.

3 min. One unit of XO activity was defined as 1 mol urate

activity was measured at 25°C on a spectrophotometer at 290 nm for

tissue.

15 min. The oxonic acid- and infarct size did not differ among the infarcted groups.

Allopurinol, ABT-627, and tempol, compared with

infarcted groups treated with vehicle, OA and ABT-627, and OA + Tem. *P < 0.05, compared with

infarcted groups treated with vehicle, OA + All, OA + ABT-627, and OA + Tem. §P < 0.05, compared with

infarcted groups treated with vehicle, OA + All, and OA + Benz.

Table 1. Cardiac morphology, hemodynamics, and uric acid concentrations at the end of study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham/Vehicle</th>
<th>Vehicle</th>
<th>OA</th>
<th>OA/All</th>
<th>OA/Benz</th>
<th>OA/ABT-627</th>
<th>OA/Tem</th>
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<tbody>
<tr>
<td>No. of rats</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>12</td>
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<tr>
<td>BW, g</td>
<td>415 ± 15</td>
<td>401 ± 21</td>
<td>395 ± 25</td>
<td>414 ± 17</td>
<td>424 ± 18</td>
<td>406 ± 28</td>
<td>415 ± 23</td>
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<td>Heart rate, beats/min</td>
<td>383 ± 18</td>
<td>405 ± 21</td>
<td>394 ± 20</td>
<td>410 ± 18</td>
<td>404 ± 22</td>
<td>407 ± 17</td>
<td>411 ± 15</td>
</tr>
<tr>
<td>LVESp, mmHg</td>
<td>119 ± 4</td>
<td>97 ± 11</td>
<td>106 ± 8</td>
<td>102 ± 6</td>
<td>105 ± 10</td>
<td>102 ± 7</td>
<td>105 ± 6</td>
</tr>
<tr>
<td>LVEDp, mmHg</td>
<td>7 ± 3</td>
<td>18 ± 2*</td>
<td>19 ± 4*</td>
<td>16 ± 5*</td>
<td>15 ± 5*</td>
<td>18 ± 4*</td>
<td>18 ± 4*</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>7,652 ± 538</td>
<td>2,954 ± 384*</td>
<td>2,748 ± 399*</td>
<td>4,012 ± 327*</td>
<td>2,598 ± 285*</td>
<td>3,924 ± 422*</td>
<td>3,963 ± 242*</td>
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<tr>
<td>−dP/dt, mmHg/s</td>
<td>6,722 ± 382</td>
<td>2,201 ± 354*</td>
<td>2,102 ± 212*</td>
<td>3,492 ± 380*</td>
<td>2,023 ± 275*</td>
<td>3,292 ± 375*</td>
<td>2,976 ± 412*</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>0</td>
<td>41 ± 4</td>
<td>42 ± 3</td>
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<td>42 ± 4</td>
<td>42 ± 4</td>
<td>40 ± 5</td>
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<td>LVW/BW, mg/g</td>
<td>2.05 ± 0.21</td>
<td>2.94 ± 0.26*</td>
<td>2.89 ± 0.25*</td>
<td>2.75 ± 0.41*</td>
<td>2.83 ± 0.25*</td>
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<td>2.95 ± 0.25*</td>
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<td>RVW/BW, mg/g</td>
<td>0.48 ± 0.06</td>
<td>0.59 ± 0.14*</td>
<td>0.68 ± 0.17‡</td>
<td>0.59 ± 0.10*</td>
<td>0.74 ± 0.08‡</td>
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<td>0.55 ± 0.12</td>
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<td>Lung weight/BW, mg/g</td>
<td>4.15 ± 0.32</td>
<td>5.07 ± 0.34†</td>
<td>5.46 ± 0.43‡</td>
<td>4.52 ± 0.32</td>
<td>5.53 ± 0.32‡</td>
<td>4.35 ± 0.39</td>
<td>4.50 ± 0.31‡</td>
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<tr>
<td>Uric acid, mg/dl</td>
<td>0.87 ± 0.11</td>
<td>1.30 ± 0.22*</td>
<td>3.12 ± 0.22‡</td>
<td>1.26 ± 0.15*</td>
<td>1.14 ± 0.19*</td>
<td>2.88 ± 0.16§</td>
<td>2.95 ± 0.24§</td>
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</table>

Values are means ± SD. All, allopurinol; Benz, benzbromarone; BW, body weight; LVESp, left ventricular end-diastolic pressure; LVEDp, left ventricular end-systolic pressure; +dP/dt and −dP/dt, maximal rate of left ventricular pressure rise and decrease, respectively; LVW, left ventricular weight; OA, oxonic acid; RVW, right ventricular weight; Tem, tempol. *P < 0.05, compared with sham. †P < 0.05, compared with infarcted groups treated with OA + All, OA + ABT-627, and OA + Tem. §P < 0.05, compared with infarcted groups treated with vehicle, OA + All, OA + ABT-627, and OA + Tem.}

Cardiac hypertrophy and fibrosis. To characterize the cardiac hypertrophy on a cellular level, morphometric analyses of LV sections from the remote zone were performed on different treatment groups (Fig. 1). Myocytes were significantly hypertrophied in the vehicle-treated infarcted group compared with those in the sham-operated group. Furthermore, oxonic acid-treated infarcted rats had a significant increase in cardiomyocyte size compared with vehicle-treated infarcted rats. Allopurinol, ABT-627, and tempol reduced cell areas by 33, 39, and 32% compared with the oxonic acid-treated infarcted group (all P < 0.05). However, rats admin-

groups to a similar extent. The infarcted groups that received ABT-627 and tempol had similar values of uric acid as the oxonic acid-treated infarcted group.

Allopurinol, ABT-627, and tempol had similar values of uric acid as the oxonic acid-treated infarcted group.
istered benzbromarone developed cardiomyocyte hypertrophy compared with allopurinol-treated group.

Fibrosis of the LV from the remote zone was examined in tissue sections after Sirius red staining, as shown in Fig. 2. Infarcted rats treated with vehicle had significantly larger areas of intense focal fibrosis compared with sham-operated rats (0.13 ± 0.05 vs. 0.03 ± 0.01%; P < 0.05). Compared with vehicle, oxonic acid-treated infarcted rats had increased fibrosis, as observed by increased collagen staining. The hyperuricemic infarcted rats showed attenuated cardiac fibrosis after administering either allopurinol, ABT-627, or tempol.

**Echocardiography.** In comparison with sham-operated animals, MI hearts showed structural changes such as increased LV diastolic and systolic diameters (Table 2; Fig. 3), consistent with LV remodeling. Echocardiography showed a significant increase in LV end-systolic diameter dimension and LV end-diastolic diameter dimension and in oxonic acid-treated infarcted rats compared with vehicle-treated infarcted rats. LV fractional shortening was significantly lower in oxonic acid-treated infarcted rats compared with vehicle-treated infarcted rats (16 ± 3 vs. 19 ± 3% in vehicle; P < 0.05). Oxonic acid significantly increased the hypertrophy of the remote myocardium (i.e., LV posterior wall; P < 0.05) but did not affect the thickness of the infarcted portion (i.e., interventricular septum). Infarcted groups treated with allopurinol, ABT-627, or tempol, but not benzbromarone, can improve LV remodeling and function. These data were corroborated by the results that +dP/dt and −dP/dt were significantly improved in the allopurinol-treated group compared with the benzbromarone-treated group, although both groups had similar uric acid reduction.

**Myocardial superoxide, free 15-F2t-isoprostane, and XO activities.** Superoxide production was significantly increased in remote LV tissues from vehicle-treated infarcted rats compared with sham and further increased in those from oxonic acid-treated infarcted rats (Fig. 4A). It was significantly decreased in allopurinol and ABT-627-treated rats to the level of sham. The chronic administration of the superoxide scavenger tempol into MI animals completely prevented the production of superoxide.

Myocardial free 15-F2t-isoprostane in vehicle-treated infarcted rats significantly increased compared with sham (P < 0.001; Fig. 4B). Oxonic acid administration further induced the overexpression of myocardial free 15-F2t-isoprostane. Myocardial free 15-F2t-isoprostane in tempol-treated infarcted rats can be reduced to the levels similar to those in the allopurinol-treated infarcted rats. However, when compared with allopurinol-treated infarcted rats, benz bromarone-treated infarcted rats had significantly higher myocardial free 15-F2t-isoprostane.

As shown in Fig. 4C, compared with that in the sham group, XO activity in the vehicle-treated infarcted group increased significantly. Oxonic acid-treated infarcted rats showed a significantly higher XO activity compared with vehicle-treated infarcted rats. However, XO activity in the allopurinol group decreased significantly compared with that in the oxonic acid- and benz bromarone-treated infarcted rats.

**DHE staining in myocardium.** DHE reacts with superoxide radicals to form ethidium bromide, which in turn intercalates with DNA to provide nuclear fluorescence as a marker of superoxide radical generation. As shown in Fig. 5, postinfarction remodeling markedly enhanced the intensity of the DHE staining in the remote zone in the vehicle-treated rats compared with sham. When compared with vehicle-treated infarcted rats, oxonic acid-treated infarcted rats had significantly increased intensity of the fluorescent signal. The hyperuricemia-induced increased intensity of the fluorescent signal can be significantly attenuated by administering with allopurinol, ABT-627, or tempol but not benz bromarone.

**ET-1 protein and mRNA expression.** Western blot shows that ET-1 levels were significantly upregulated 3.3-fold in the remote zone in the vehicle-treated infarcted rats than in sham-operated rats (P < 0.0001; Fig. 6). When compared with vehicle-treated infarcted rats, oxonic acid-treated infarcted rats had significantly higher ET-1 levels at the remote zone (2.3 ± 0.6 in vehicle vs. 2.7 ± 0.5; P < 0.05). The hyperuricemia-induced increased ET-1 expression can be significantly attenuated by administering with either allopurinol or tempol but not benz bromarone. The tempol-treated infarcted rats showed significant reduction of ET-1 expression compared with the infarcted rats treated with oxonic acid alone.

**DISCUSSION**

Our present study shows for the first time that modest hyperuricemia markedly exacerbated ventricular remodeling probably through a ROS-dependent ET-1 pathway in infarcted rats with experimentally induced hyperuricemia. These results were consistent with the effects of hyperuricemia in infarcted rats, as documented structurally by increase in cardiomyocyte size and cardiac fibrosis; molecularly by myocardial ET-1 protein and mRNA levels; biochemically by myocardial superoxide, 15-F2t-isoprostane, and XO activities; and functionally by impairment of heart contractility. Chronic scavenging of superoxide with tempol or an ETA receptor blocker, ABT-627, was able to prevent ventricular remodeling induced by hyperuricemia despite serum uric acid remaining elevated. However, reduction of increased uric acid levels by uricosuric therapy (i.e., without inhibition of the XO pathway) has no effect on ROS levels and ventricular remodeling. Thus uric acid itself would simply be a marker rather than the culprit per se.

The influence of elevated uric acid on ventricular remodeling has never been thoroughly examined before. The observation that oxonic acid-treated rats had unfavorable effects in ventricular remodeling does not prove that uric acid is responsible for the impairment of ventricular remodeling, because it is possible that the oxonic acid could be causing the impaired ventricular remodeling independent of its ability to raise uric acid. To determine whether the impaired ventricular remodeling was because of the hyperuricemia and not a nonspecific effect of oxonic acid, rats on oxonic acid received either a XO inhibitor, allopurinol, or a uricosuric agent, benz bromarone. The effect of hyperuricemia and its treatment on attenuated ventricular remodeling was supported by three lines of evidence: 1) hyperuricemic rats have a further increase in myocardial superoxide and 15-F2t-isoprostane compared with nor-
Fig. 2. A–G: representative sections from the remote zone with 
Sirius red staining (red, magnification: ×400) at 4 wk after 
within the left ventricle (LV) is reduced after allopurinol, 
ABT-627, or tempol was administered. Line length corresponds 
to 50 μm. Bottom: LV collagen area fraction (%). Each column 
and bar represents means ± SD. Number of animals in each 
group is indicated in parentheses. *P < 0.05, compared with 
sham, vehicle-, OA + All-, OA + ABT-627-, and OA + 
Tem-treated groups. †P < 0.05, compared with infarcted 
groups treated with OA + All, OA + ABT-627, and OA + 
Tem.
mouricemic rats after MI. Our present study showed that oxidative stress as assessed with a DHE staining and myocardial XO activities is increased in the remote myocardium after MI. Our results were consistent with the findings of Stull et al. (39), showing that myocardial superoxide production was significantly increased 4 wk after MI. 2) Treatment with inhibition of ROS and ET-1 can attenuate ventricular remodeling. ROS have been implicated as an important contributing factor in LV remodeling.

Table 2. **Echocardiographic findings**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham/Vehicle</th>
<th>Vehicle</th>
<th>OA</th>
<th>OA/All</th>
<th>OA/Benz</th>
<th>All/ABT-627</th>
<th>All/Tem</th>
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<tr>
<td>Interventricular septum, mm</td>
<td>1.5 ± 0.1</td>
<td>0.6 ± 0.2*</td>
<td>0.7 ± 0.2*</td>
<td>0.6 ± 0.2*</td>
<td>0.6 ± 0.1*</td>
<td>0.7 ± 0.2*</td>
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</tr>
<tr>
<td>LVEDD, mm</td>
<td>5.8 ± 0.2</td>
<td>8.5 ± 0.2*†</td>
<td>9.1 ± 0.2*‡</td>
<td>7.5 ± 0.2*</td>
<td>8.8 ± 0.2*‡</td>
<td>7.4 ± 0.1*</td>
<td>7.6 ± 0.2*</td>
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<tr>
<td>LVESD, mm</td>
<td>3.6 ± 0.1</td>
<td>6.9 ± 0.2*†</td>
<td>7.6 ± 0.2*‡</td>
<td>5.3 ± 0.2*</td>
<td>7.4 ± 0.1*‡</td>
<td>5.3 ± 0.3*</td>
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</tr>
<tr>
<td>LV posterior wall, mm,</td>
<td>1.5 ± 0.1</td>
<td>1.8 ± 0.2*†</td>
<td>2.1 ± 0.1*‡</td>
<td>1.7 ± 0.1*</td>
<td>2.2 ± 0.2*‡</td>
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</tr>
<tr>
<td>FS, %</td>
<td>38 ± 2</td>
<td>19 ± 3*†</td>
<td>16 ± 3*‡</td>
<td>28 ± 3*</td>
<td>16 ± 2*‡</td>
<td>29 ± 3*</td>
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Values are means ± SD. Abbreviations are as in Table 1. FS, fractional shortening; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension. *P < 0.05, compared with the sham group. †P < 0.05, compared with infarcted groups treated with OA + All, OA + ABT-627, and OA + tempol. ‡P < 0.05, compared with infarcted groups treated with vehicle, OA + All, OA + ABT-627, and OA + Tem.

Fig. 3. Representative M-mode image reveals a hypokinetic-to-akinetic anterior wall and LV dilation in the infarcted hearts (B–G) in contrast to normal anterior wall motion in a sham-operated heart (A). IVS, interventricular septum; PW, posterior wall.
remodeling after MI (15). Both tempol and ABT-627 ameliorated unfavorable effects of hyperuricemia in this rat model by improving ventricular remodeling through its antioxidant properties, and it had no direct effects on the levels of uric acid. Furthermore, tempol administration can attenuate ET-1 expression, which may suggest that ROS play a role in regulating ET-1. These results indicate that the ventricular remodeling after infarction is subject to ROS-ET-1 regulation. 3) The exacerbation of post-MI LV remodeling was inhibited by an inhibitor of XO activation, allopurinol, but not a uricosuric agent, benzbromarone, although both had similar reduction of uric acid. Previous studies (32) have shown that benzbromarone can inhibit XO activity. However, this is unlikely, because benzbromarone as a XO antagonist required higher therapeutic concentrations than those reported in this study. XO activity was increased, and ROS production was enhanced in the remote myocardium. The protective effects associated with allopurinol treatment in this model provide additional support for the role of XO in the prevention of ventricular remodeling post-MI.

The relationship between serum uric acid level and XO activity has been debated, although Tan and colleagues (31, 41) have shown that uric acid negatively feedbacked on XO activity in an acute elevation of uric acid. However, it has to be recognized that not only the total increase in systemic uric acid but also the duration of its elevation is of significant importance in eliciting uric acid-related action. For example, endothelial progenitor cell mobilization was detectable only with acute increases of uric acid, whereas a chronic and prolonged hyperuricemia suppresses this effect (30). Thus whether the findings of Tan and colleagues (31, 41) in acute hyperuricemic models can apply to our chronic settings remained unclear. In contrast, uric acid can have positive feedback on XO activity. Uric acid has been recognized to have an important role in innate immunity and especially the activation of dendritic cells and antigen-presenting cells to endogenous antigens (37). Furthermore, Kono et al. (17) have shown that uric acid as a proinflammatory molecule contributes significantly to the cell death-induced inflammatory responses in vivo. Inflammation has been shown to increase XO activities (11). Thus it is not surprising that XO activities were greater in hyperuricemic rats compared with those in normouricemic rats. Indeed, our results in the postinfarcted hearts were consistent with previous findings of Gourine et al. (9), showing that elevated uric acid levels were associated with increased XO activities in myocardial tissue. Furthermore, the levels of uric acid formed in the uricase-inhibition reaction of the present work did not seem to be inhibitory. Previously reported inhibition of XO by uric acid was observed at higher concentrations (31, 41).

Available data suggest that uric acid is not necessarily an antioxidant and, depending on the chemical milieu, may become a prooxidant. Previous studies (2, 38) have shown that uric acid is an effective inhibitor of the formation of superoxide and hydrogen peroxide. In humans, uric acid is maintained at a concentration close to maximum solubility and is the most important aqueous antioxidant, contributing near two-thirds of total plasma antioxidant capacity (38). Uric acid is oxidized to allantoin in a process that scavenges hydroxyl radicals, lipid hydroperoxide radicals, singlet oxygen, and oxo-heme oxidants while inhibiting lipid peroxidation in myocardium (2). In contrast, uric acid has been shown to induce a marked oxidative burst at physiological concentrations (49). Oxidative effects of uric acid were blocked by probenecid, which is known to prevent entry of uric acid into vascular cells (12). Thus uric acid, while being an antioxidant in the extracellular environment, has direct prooxidative effects once it gains entry into intracellular sites (35). Thus, although both benzbromarone and allopurinol had similar reduction of serum uric acid, allopurinol may be more effective at lowering intracellular uric acid by inhibiting intracellular XO activities (8). It is not surprising to know that the exacerbation of ROS-related LV remodeling after infarction was inhibited by an XO inhibitor, allopurinol, but not a uricosuric agent, benzbromarone.

Exactly how inhibition of superoxide leads to attenuated ET-1 expression cannot be determined from this study. The ET-1 promoter contains a number of activator protein-1, which
Fig. 5. A–G: detection of superoxide in myocardium by dihydroethidium (DHE) staining. Compared with sham, the DHE fluorescence intensity in the myocardium of the vehicle-treated infarcted group was significantly increased. A: sham. B: vehicle. C: OA. D: OA + All. E: OA + Benz. F: OA + ABT-627. G: OA + Tem. Bar = 50 μm. Bottom: DHE staining (%) in the remote zone. Each column and bar represents means ± SD. Number of animals in each group is indicated in parentheses. Arb. U, arbitrary units. *P < 0.05, compared with sham, vehicle-, OA + All-, OA + ABT-627-, and OA + Tem-treated groups. †P < 0.05, compared with infarcted groups treated with OA + All, OA + ABT-627, and OA + Tem.
High-energy phosphates ATP and PCr in allopurinol-treated hearts (29). In contrast, benzbromarone has been shown to inhibit mitochondrial β-oxidation (13). Thus the improvement in ventricular remodeling could be secondary to a direct effect of allopurinol on improvement in myocardial energetic efficiency (4). Second, concerning the mechanisms involved in the attenuation of allopurinol-induced hypertrophy, it is attractive to also consider a potential role of angiotensin II, a potent growth-promoting peptide. Allopurinol has been shown to inhibit angiotensin II-induced increase in collagen production (24). We cannot exclude the possibility of allopurinol-induced inhibition of angiotensin II in this process. Complex interactions among ET-1, angiotensin II, and allopurinol exist that could affect cardiac hypertrophy.

Study limitations. There are some limitations in the present study that have to be acknowledged. Our finding in rats cannot necessarily be extrapolated to humans with MI. First, given the large number of potential ROS generators, it is perhaps surprising that the elimination of a single one like XO had any effect at all. It is possible that uric acid plays a role that is different from other ROS generators. XO might provide additive or synergistic effects with other ROS generators. It would also not be surprising if different tissues have different contents of ROS generators, in which case a specific ROS generator such as XO might play a greater or lesser role in different tissues. We cannot rule out the participation of different sources of oxygen radicals in this pathlogy. Second, the source of increased ROS cannot be determined from this study. A number of cell types not normally present in the myocardial tissues to any substantial degree may also produce ROS and contribute to increased levels of tissue ROS, such as endothelium (2) and fibroblasts (5). However, DHE staining resided mainly in the cardiomyocytes compared with vascular smooth muscle and endothelial cells, suggesting that the cardiomyocyte is one of the main sources for cardiac ROS. Finally, there are well-established interspecies differences in intrinsic levels of myocardial XO activity (42). Rats used in this study have relatively high levels of myocardial XO activity, whereas the is subjected to redox regulation (14). Uric acid has been shown to significantly increase activator protein-1-mediated reporter activity in neonatal rat cardiac fibroblasts (5). Moreover, pretreated cells with antioxidants attenuated the uric acid-stimulated activator protein-1-mediated reporter activity. Mutational analysis of the ET-1 gene promoter showed that activator protein-1-mediated reporter activity is subjected to redox regulation (14). Uric acid has been shown to significantly increase activator protein-1-mediated reporter activity in neonatal rat cardiac fibroblasts (5). Moreover, pretreated cells with antioxidants attenuated the uric acid-stimulated activator protein-1-mediated reporter activity. Mutational analysis of the ET-1 gene promoter showed that activator protein-1-mediated reporter activity is subjected to redox regulation (14).

The benefit of allopurinol identified here, namely improvement of ventricular remodeling, contrasts with the findings of Stull et al. (39), showing no structural changes in allopurinol-treated infarcted mice 4 wk after MI. The discrepancy may be explained, at least in part, by a massive infarct size (51 ± 4%) in the former study. Previous studies (1) have shown that massive infarction resulted in increased LV end-systolic volume, which cannot be ameliorated after intervention such as revascularization. Indeed, our results were consistent with the findings of Xiao et al. (47), showing an attenuated LV dilation after adding allopurinol in rats with 30% of infarct size.

Other mechanisms. Although the present study suggests that the mechanisms of allopurinol-induced attenuation of ventricular remodeling may be related to attenuated ROS-dependent ET-1 expression, other potential mechanisms need to be studied. First, allopurinol may have had effects other than decreasing XO-mediated oxygen metabolite production. Allopurinol may promote purine salvage after an ischemic insult by inhibiting purine catabolism, which subsequently increased in the high-energy phosphates ATP and PCr in allopurinol-treated hearts (29). In contrast, benzbromarone has been shown to inhibit mitochondrial β-oxidation (13). Thus the improvement in ventricular remodeling could be secondary to a direct effect of allopurinol on improvement in myocardial energetic efficiency (4). Second, concerning the mechanisms involved in the attenuation of allopurinol-induced hypertrophy, it is attractive to also consider a potential role of angiotensin II, a potent growth-promoting peptide. Allopurinol has been shown to inhibit angiotensin II-induced increase in collagen production (24). We cannot exclude the possibility of allopurinol-induced inhibition of angiotensin II in this process. Complex interactions among ET-1, angiotensin II, and allopurinol exist that could affect cardiac hypertrophy.

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activity in humans is comparatively low (6). Besides, rodents have significantly lower uricemia as a result of the presence of functional uricase, the enzyme that degrades uric acid into allantoin and that is not expressed in humans as a result of a mutation (48). Thus our finding cannot necessarily be extrapolated to species with comparatively low activities of XO. It is not surprising that a clinical trial [Oxypurinol Therapy for Congestive Heart Failure (OPT-CHF)] designed to demonstrate the efficacy of long-term oxypurinol treatment in heart failure patients showed no therapeutic benefit in terms of a 

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mg/dl (10). These findings are difficult to interpret. Perhaps data from the OPT-CHF study support these seemingly competing views; benefit from uric acid reduction may only be seen in those with serum uric acid high enough to harm platelet and endothelial function while uric acid reduction in those with lower levels may compromise plasma oxidant activity such that this could be of detriment.

**Conclusions.** These data show that mild hyperuricemia is associated with increased myocardial oxidative stress, which contributes to the development of ventricular remodeling. Moreover, inhibition of superoxide and ET-1 attenuated the adverse effects induced by increased uric acid. Further studies will be necessary to determine the relevance of these findings in the setting of hyperuricemia-associated ventricular remodeling in humans.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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