Allopurinol Enhances the Contractile Response to Dobutamine and Exercise in Dogs With Pacing-Induced Heart Failure

Tomohiko Ukai, MD, PhD; Che-Ping Cheng, MD, PhD; Hideo Tachibana, MD, PhD; Akihiko Igawa, MD, PhD; Zhu-Shan Zhang, MD, PhD; Heng-Jie Cheng, MD, PhD; William C. Little, MD

Background—Superoxide (O$_2^-$) generated by enhanced xanthine oxidase (XO) activity may contribute to the increased myocardial oxidative stress in heart failure (CHF). Because blocking XO with allopurinol augments myofilament Ca$^{2+}$ sensitivity in reperfusion injury and CHF, we hypothesized that it may improve adrenergic inotropic responsiveness in CHF.

Methods and Results—We studied the effect of allopurinol on the contractile response to dobutamine and exercise in 7 chronically instrumented conscious dogs before and after producing CHF by rapid pacing. Left ventricular (LV) contractile performance was measured by the slopes of the LV end-systolic pressure-volume relation (E$_{ES}$) and stroke work–end-diastolic volume relation (M$_{SW}$). Before CHF, allopurinol produced no change in LV contractile performance and did not alter the response to dobutamine or exercise. After CHF, allopurinol produced significant (P<0.05) increases in E$_{ES}$ (5.0±0.6 versus 3.3±0.6 mm Hg/mL) and M$_{SW}$. Dobutamine and allopurinol produced greater increases in E$_{ES}$ (5.4±0.6 versus 7.4±0.6 mm Hg/mL) and M$_{SW}$ (60.1±7.4 versus 73.7±4.4 mm Hg) than did dobutamine alone. After allopurinol, dP/dt$_{max}$, stroke volume, and M$_{SW}$ were higher during CHF exercise. LV diastolic pressures were lower during CHF exercise after allopurinol.

Conclusions—Allopurinol has no discernable effects on LV contractile function or adrenergic responsiveness in normal, conscious animals. In pacing-induced CHF, however, allopurinol improves LV systolic function at rest and during adrenergic stimulation and exercise. (Circulation. 2001;103:750-755.)

Key Words: antioxidants ■ exercise ■ heart failure ■ receptors

Myocardial oxidative stress due to increased production of reactive oxygen free radicals may play an important role in the development and progression of myocardial dysfunction in heart failure (CHF). Neurohormonal and inflammatory factors in CHF contribute to the enhanced oxidative stress. In addition, there is increased production of the reactive oxygen radical superoxide (O$_2^-$) by myocardial mitochondria in CHF. Xanthine oxidase (XO) may also contribute to myocardial oxidative stress in CHF. XO forms O$_2^-$ as it catalyzes the terminal steps in the breakdown of purines to uric acid. Ekelund et al$^{7}$ recently demonstrated a 4-fold increase in myocardial XO in dogs with pacing-induced CHF. Patients with decompensated heart failure have elevated serum uric acid$^8$ consistent with increased XO activity in CHF.

O$_2^-$ generated by enhanced XO activity plays an important role in reperfusion injury by damaging or functionally modifying contractile proteins, resulting in reduced calcium sensitivity.$^9$–$^{11}$ Blocking XO with allopurinol enhances calcium sensitivity in rat stunned trabeculae.$^{12}$ Similarly, blocking the increased myocardial XO activity in animals with pacing-induced CHF enhances baseline left ventricular (LV) contractile performance while decreasing myocardial oxygen consumption.$^7$ These observations suggest that blocking XO may enhance myocardial calcium sensitivity in CHF.

$\beta$-Adrenergic stimulation produced pharmacologically or during exercise increases myocardial contractile performance. This is partially due to an enhancement of the calcium transient. The response to $\beta$-adrenergic stimulation is reduced in CHF because of receptor downregulation and uncoupling.$^{13}$ The reduced $\beta$-adrenergic responsiveness may play an important role in an abnormal response to exercise in CHF.$^{14}$

We hypothesize that if blocking XO increases myocardial calcium sensitivity in CHF, it should enhance the contractile response to pharmacologically produced $\beta$-adrenergic stimulation as well as to the endogenous $\beta$-adrenergic stimulation that occurs during exercise. Accordingly, we studied the contractile response to dobutamine and exercise in dogs before and after producing CHF by tachycardia pacing. Our
TABLE 1. Effects of Allopurinol and Combination of Dobutamine and Allopurinol on Steady-State Hemodynamic Data Before and After CHF

<table>
<thead>
<tr>
<th></th>
<th>Before CHF</th>
<th>After CHF</th>
<th>AL + Dobutamine</th>
<th>After CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Allopurin</td>
<td>Dobutamine</td>
<td>Control</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>107 ± 12</td>
<td>113 ± 13</td>
<td>124 ± 11*</td>
<td>117 ± 8</td>
</tr>
<tr>
<td>dP/dt\text{max}, mm Hg/s</td>
<td>2418 ± 370</td>
<td>2469 ± 378</td>
<td>3045 ± 527*</td>
<td>1302 ± 240</td>
</tr>
<tr>
<td>dP/dt\text{min}, mm Hg/s</td>
<td>-1972 ± 426</td>
<td>-1933 ± 388</td>
<td>-2208 ± 382</td>
<td>-2203 ± 448</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>8.2 ± 5.9</td>
<td>6.8 ± 6.1</td>
<td>7.9 ± 5.3</td>
<td>7.5 ± 4.9</td>
</tr>
<tr>
<td>LV end-systolic pressure, mm Hg</td>
<td>105 ± 6.0</td>
<td>101 ± 5.2</td>
<td>103 ± 7.9</td>
<td>102 ± 8.5</td>
</tr>
<tr>
<td>Min LV pressure, mm Hg</td>
<td>1.6 ± 1.7</td>
<td>0.9 ± 2.1</td>
<td>-2.1 ± 2.7*</td>
<td>-1.7 ± 3.1</td>
</tr>
<tr>
<td>Mean LA pressure, mm Hg</td>
<td>6.1 ± 1.1</td>
<td>4.7 ± 2.1</td>
<td>4.5 ± 1.7</td>
<td>5.1 ± 1.8</td>
</tr>
<tr>
<td>LV end-diastolic volume, mL</td>
<td>44.2 ± 10.0</td>
<td>44.1 ± 9.7</td>
<td>43.7 ± 9.2</td>
<td>43.8 ± 8.9</td>
</tr>
<tr>
<td>LV end-systolic volume, mL</td>
<td>29.5 ± 9.7</td>
<td>29.1 ± 8.0</td>
<td>27.3 ± 7.7*</td>
<td>26.9 ± 8.2</td>
</tr>
<tr>
<td>Stroke work, mm Hg · mL</td>
<td>1479 ± 237</td>
<td>1514 ± 330</td>
<td>1717 ± 312*</td>
<td>1887 ± 317</td>
</tr>
<tr>
<td>τ, ms</td>
<td>0.071 ± 0.017</td>
<td>0.062 ± 0.015</td>
<td>0.053 ± 0.014*</td>
<td>0.061 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>31.9 ± 2.5</td>
<td>32.7 ± 3.6</td>
<td>28.9 ± 3.4*</td>
<td>27.6 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>39.1 ± 6.1</td>
<td>35.0 ± 4.0†</td>
<td>35.1 ± 3.5*</td>
<td>31.6 ± 4.3†</td>
</tr>
</tbody>
</table>

TSR indicates total systemic resistance. Values are mean ± SD (n = 7 dogs).
*P < 0.05, dobutamine vs corresponding control value.
†P < 0.05, allopurinol vs corresponding control value.
‡P < 0.05, allopurinol plus dobutamine vs corresponding dobutamine value.

Results

provide insight into a mechanism of contractile dysfunction in CHF and suggest a potential method of enhancing the β-adrenergic responsiveness of the failing myocardium.

Methods

Instrumentation
Seven healthy, adult, heartworm-negative mongrel dogs (25 to 35 kg) were instrumented to measure 3 LV internal dimensions and LV and left atrial (LA) pressures. Hydraulic occluders were placed around the venae cavae by the technique that we described previously.15,16

Drug Preparation
Allopurinol (300 mg, Sigma) was dissolved in 100 mL of normal saline after slight heating and alkalinization with NaOH.

Experimental Protocol

Studies Before CHF at Rest
Studies were begun after full recovery from the instrumentation. To obtain baseline values, data were initially recorded with the un paced dogs standing quietly on a motorized treadmill. Three sets of variably loaded LV pressure-volume (P-V) loops were generated by sudden, transient occlusion of the cavae as we described previously.14 The effects of the following interventions were studied on separate days in random order with the animals allowed to equilibrate for 2 days between studies.

1. Infusion of an inhibitor of XO, allopurinol (0.2 mg · kg⁻¹ · min⁻¹ IV), for 40 minutes.
2. Infusion of dobutamine (8 μg · kg⁻¹ · min⁻¹ IV).
3. Infusion of allopurinol for 40 minutes, followed by an infusion of dobutamine.
4. The effect of treadmill exercise. We collected the data at 3 and 4 mph and at the maximum tolerated level of steady-state exercise as described.14 After a 30-minute rest period, allopurinol was administered. Forty minutes after allopurinol administration, the exercise protocol was repeated.

Induction of CHF

After completion of the initial studies, rapid right ventricular pacing was initiated. The pacing rate was adjusted with an external magnetic control unit to 200 to 240 bpm as described. After pacing for 3 to 4 weeks, when the LV end-diastolic pressure during the nonpaced period had increased by >15 mm Hg over the prepacing control level, CHF data were obtained.

Studies After CHF

During the stable CHF period, we conducted the same studies as before CHF. Before each study, the pacemaker was turned off, and the animal was allowed to equilibrate for 1 hour. After the study, the rapid pacing was resumed.

Data Processing and Analysis

As previously described,16 LV volume was calculated as a modified general ellipsoid. The rate of LV relaxation was analyzed by determining the exponential time constant (τ) of the isovolumic fall of LV pressure.

Analyses of LV P-V Loop During Caval Occlusion

As previously described,17 we determined the LV end-systolic pressure (P\text{es})–end-systolic volume (V\text{es}) relation, the stroke work (SW)–end-diastolic volume (V\text{ed}) relation, and the dP/dt\text{max}–V\text{ed} relation from the variably loaded beats produced by transient caval occlusion. Because we could not perform caval occlusions during exercise, we assessed LV contractile performance during exercise by calculating M\text{aw} assuming that the volume axis intercept was unchanged.18

Statistical Analysis

Data are expressed as mean ± SD. Multiple comparisons were performed by ANOVA. When a significant overall effect was present, intergroup comparisons were performed with a Bonferroni correction for multiple comparisons. The level of significance was P < 0.05. We evaluated the interaction of allopurinol with dobutamine and exercise by ANOVA of 2 factors with replication.

Results

Effects of Allopurinol Administered Before and After CHF at Rest

Before CHF, allopurinol produced no changes in steady-state hemodynamic parameters (Table 1) or in any of the LV P-V measures of LV contractile performance (Table 2, Figure 1). After CHF, allopurinol caused increases in stroke volume and
Effects of Combination of Allopurinol and Dobutamine

Before CHF, allopurinol had no discernable effect on the response of LV contractile performance to dobutamine (Table 2, Figure 1). After CHF, the response to dobutamine alone was blunted. After allopurinol, however, dobutamine produced greater increases in stroke volume and cardiac output (Table 1). The contractile response to dobutamine was also enhanced by pretreatment with allopurinol (Table 2, Figure 2). Two-factor ANOVA demonstrated no significant interaction between allopurinol and dobutamine.

Effects of Exercise After Allopurinol

Before CHF, allopurinol did not significantly alter the response to exercise. The response of LV systolic performance to exercise was reduced after CHF, and minimum LV pressure and τ increased (rather than the normal decrease during exercise) during CHF exercise. After allopurinol, dP/dtmax, dV/dtmax, and M₁SW were greater during exercise, and LV diastolic pressure and τ were lower than during control CHF exercise (Table 3, Figures 3 and 4). There was no significant interaction between exercise and allopurinol.

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**TABLE 2. Effects of Allopurinol and Combination of Dobutamine and Allopurinol on LV P-V Relations Before and After CHF**

<table>
<thead>
<tr>
<th>Relation</th>
<th>Before CHF</th>
<th>Allopurinol</th>
<th>Dobutamine</th>
<th>Allopurinol and Dobutamine</th>
<th>After CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PES-VES Relation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EES, mm Hg/mL</td>
<td>5.6±1.1</td>
<td>5.8±1.0</td>
<td>9.4±1.7*</td>
<td>9.1±1.5</td>
<td>3.3±0.7</td>
</tr>
<tr>
<td>V₀,ES, mL</td>
<td>7.4±2.6</td>
<td>7.7±2.2</td>
<td>13.3±1.5</td>
<td>12.7±1.5</td>
<td>-1.4±2.6</td>
</tr>
<tr>
<td>V₁₀₀₀,ES, mL</td>
<td>29.6±4.5</td>
<td>28.4±2.7</td>
<td>25.9±2.6*</td>
<td>25.5±2.7</td>
<td>34.7±5.6</td>
</tr>
<tr>
<td>dP/dtmax-VES relation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dE/dtmax, mm Hg·s⁻¹·mL⁻¹</td>
<td>62.8±10.6</td>
<td>61.1±8.8</td>
<td>74.9±10.8*</td>
<td>75.6±10.9</td>
<td>29.2±4.3</td>
</tr>
<tr>
<td>V₁₀,dp/dt, mL</td>
<td>1.1±3.4</td>
<td>1.4±3.6</td>
<td>1.9±1.9</td>
<td>1.6±1.3</td>
<td>6.3±2.3</td>
</tr>
<tr>
<td>V₁₀₀₀,dp/dt, mL</td>
<td>17.0±3.2</td>
<td>17.8±3.0</td>
<td>12.8±6.1*</td>
<td>14.8±9.3</td>
<td>40.5±7.0</td>
</tr>
<tr>
<td><strong>SW-VES relation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W₀, mm Hg</td>
<td>75.0±4.3</td>
<td>73.2±4.5</td>
<td>84.4±2.7*</td>
<td>90.3±5.1</td>
<td>44.8±4.1</td>
</tr>
<tr>
<td>V₀,SW, mL</td>
<td>23.8±3.8</td>
<td>22.6±3.2</td>
<td>21.1±2.7</td>
<td>20.7±3.0</td>
<td>25.1±5.1</td>
</tr>
<tr>
<td>V₁₀₀₀,SW, mL</td>
<td>37.5±3.9</td>
<td>36.6±3.7</td>
<td>33.0±2.8*</td>
<td>33.0±2.8</td>
<td>49.1±4.7</td>
</tr>
</tbody>
</table>

| V₀,dP/dt, mL             |            |             |            |                             |           |
| V₁₀₀,ES, mL              |            |             |            |                             |           |

V₀,ES indicates intercept with volume axis; V₁₀₀₀,ES, volume associated with P₀ of 100 mm Hg; dE/dtmax, slope of dP/dtmax-VES relation; V₀,SW, intercept with volume axis; and V₁₀₀₀,SW, volume associated with SW of 1000 mm Hg·mL. Values are mean±SEM (n=7 dogs).

*P<0.05, dobutamine vs corresponding control value.
†P<0.05, allopurinol vs corresponding value.
‡P<0.05, allopurinol plus dobutamine vs corresponding dobutamine value.

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**Figure 1.** LV P-V loops produced by transient caval occlusion during control and after administration of allopurinol. Data are shown before and after CHF in same animal. LV end-systolic P-V relations are indicated by lines. Under normal conditions, allopurinol had no discernable effect. After CHF, allopurinol produced leftward shift with increase in slope. This indicates that allopurinol improved LV contractile performance after CHF.

**Figure 2.** Effects of dobutamine alone and after administration of allopurinol on variably loaded LV P-V loops. Data are shown before and after CHF in same animal. Under normal conditions, allopurinol had no effect on response to dobutamine. After CHF, dobutamine produced a greater increase in slope of end-systolic P-V relation when combined with allopurinol.
and exercise. This indicates that an XO-mediated effect may

arterial oxygen tension [17]. Blocking XO with allopurinol enhances calcium sensitivity in stunned rat trabeculae. Ekelund et al. recently demonstrated a 4-fold increase in myocardial XO in dogs with pacing-induced CHF. They found that blocking XO activity in these animals with allopurinol enhanced baseline LV contractile performance while decreasing myocardial oxygen consumption. Our results confirm their observations and extend them by demonstrating an enhanced response to β-adrenergic stimulation and exercise. This indicates that an XO-mediated effect may
Our results show that allopurinol may allow use of lower doses of dobutamine by increasing β-adrenergic responsiveness. This might allow achievement of similar hemodynamic improvement with less risk, especially because allopurinol decreases myocardial oxygen requirements. It is also possible that allopurinol, by increasing the total inotropic response, might increase the risk of dobutamine. In addition, we observed that allopurinol enhanced LV systolic and diastolic performance during exercise suggests that it might improve exercise tolerance in CHF.

We observed that after CHF, allopurinol produced slight arterial vasodilatation, increased the rate of LV isovolumic pressure fall, and lowered LV minimal pressure. In addition, the vasodilatory and lusitropic effects of dobutamine and exercise were greater after allopurinol. These effects are different from those observed by Ekelund et al., who found no lusitropic or vasodilatory effect of allopurinol after CHF.

Several limitations of our study should be considered. Although we studied an animal model of CHF (pacing tachycardia) that reproduces many of the functional and neurohormonal features of clinical CHF, we cannot be certain that our results apply to CHF of other causes. In addition, we studied the acute effects of allopurinol. We do not know the effect of prolonged treatment with allopurinol.

We studied the effect of allopurinol on the response to 1 dose of dobutamine. Thus, we did not define the adrenergic dose-response curve. Although LV contractile performance was significantly greater when dobutamine was combined with allopurinol, we observed no significant interaction between dobutamine and allopurinol. This suggests that allopurinol may produce a parallel upward shift of the adrenergic dose-response curve without a change in slope. The response to graded exercise (Figure 4) is also consistent with a parallel shift, indicating that the increment in contractile function produced by allopurinol at rest also occurs during adrenergic stimulation and exercise.

Finally, our study does not define the mechanism of action of allopurinol in CHF. It is possible that its actions are mediated by effects other than reducing the generation of O$_2^-$.

In conclusion, we found that allopurinol has no discernable effects on normal LV contractile function or β-adrenergic responsiveness in conscious animals. In pacing-induced CHF, however, allopurinol decreases LV contractile dysfunction, enhances β-adrenergic responsiveness, and improves the LV systolic and diastolic response to exercise.

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References
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