Xanthine oxidase inhibitors improve energetics and function after infarction in failing mouse hearts

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Submitted 4 August 2005; accepted in final form 6 September 2005


Abnormalities in energy metabolism after MI include reductions in ATP, phosphocreatine (PCr), and in the activity of the creatine kinase (CK) reaction, the primary energy reserve reaction of the heart (20, 21, 29, 31). Inhibitors of CK significantly increase mortality after experimental infarction (16). The reduction in cardiac PCr-to-ATP ratio (PCr/ATP) in experimental postinfarction remodeling is similar to that observed in human heart failure, which, in turn, correlates with clinical severity and predicts overall and cardiovascular mortality (4, 10, 15, 31, 34, 49). Taken together, the energetic consequences of post-MI remodeling are similar to those of heart failure and offer an additional potential mechanism that may contribute to progressive dysfunction and geometric changes.

Xanthine oxidase (XO) is important in purine metabolism, and its expression and activity are increased in heart failure (1). XO is also a major source of free radicals, such as superoxide, that can impair energy metabolism and reduce energetic efficiency (7, 11). In nonischemic experimental and human heart failure, inhibition of XO improves mechanoenergetic coupling by improving contractile performance relative to a reduced energetic demand (7, 11). Targeted XO blockade impacts on the progression of postischemic cardiomyopathy in mice (43) and attenuates left ventricular (LV) remodeling processes after experimental MI (12). Despite the evidence for improved mechanoenergetic coupling with XO inhibition in nonischemic heart failure, the metabolic and contractile effects of XO inhibition on postinfarction remodeling and the effects of XO inhibitors (XOIs) on depressed energetics in failing hearts have not been characterized.

There were two aims to this study. The first aim was to determine the extent to which geometric, contractile, and metabolic remodeling occur in vivo after nonreperfused MI in the mouse. The second aim was to test the hypothesis that XOIs improve bioenergetics and contractile function in the failing heart. This is based on the mechanism observed in nonischemic heart failure where XOIs improve mechanoenergetic coupling by improving contractile performance relative to a reduced energetic demand, such that improved energetics, as indexed by the cardiac PCr/ATP, would be expected to be associated with improved contractile function.

MATERIALS AND METHODS

All procedures and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University.

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MI in mice. Anesthesia was induced in adult mice (20–30 g) by inhalation of methoxyflurane and maintained with intraperitoneal injection of etomidate (20 mg/kg) and with subcutaneous buprenorphine (0.24 mg/kg). Additional doses of etomidate were administered as needed. Mice were intubated and ventilated with a custom-made ventilator, and the body temperature was maintained constant as monitored with a rectal probe. A left thoracotomy and pericardiotomy were performed, and the left main coronary artery was completely ligated with suture. After verification that coronary occlusion had occurred by blanching of the tissue distal to the suture, the ribs were closed with suture and the mice recovered. Additional doses of buprenorphine (0.96 mg/kg) were administered to limit discomfort. Immediately after MI surgery, XO-inhibited mice received either allopurinol (0.5 mM) or oxypurinol (1 mM) in the drinking water, whereas control animals had neither. These concentrations were previously shown to inhibit XO in this model (43). Because few pharmacological agents are completely specific, we studied both allopurinol and oxypurinol to increase the likelihood that any observed metabolic or contractile effects were due to XO inhibition and not due to another effect of one agent. All animals underwent MRI and magnetic resonance (MR) spectroscopy (MRS) studies 4 wk after surgery.

MRI and MRS. Experiments were performed by using a General Electric Omega NMR spectrometer and Bruker Medical BioSpec Spectrometer (Bruker BioSpin) equipped with a 4.7 T/40 cm Oxford magnet and a 15 cm (ID) actively shielded Accustar gradient set (8, 47).

Mice were anesthetized with 1% isoflurane in oxygen (1 l/min) delivered through a nose cone and placed in a custom-constructed 1H coil with the heart centered over the 31P coil (8, 47) on a flat Plexiglas platform with temperature control (37 ± 1°C). The mice were rotated to the left so that the uninvolved septum was centered over the surface coil, thus minimizing contributions from the infarcted lateral wall. Single-lead ECG was recorded from platinum electrodes attached to each animal’s extremities and was used to trigger the MRI acquisitions using commercial software (Small Animal Monitoring and Gating System SA Instrument, Stony Brook, NY).

High-resolution, spin-echo transverse 1H MR images (echo time, 11 ms; recycle time, 500 ms; slice thickness, 2 mm; field of view, 32 mm; and acquisition time, 2 min) were obtained to define the regions of interest perpendicular to the plane of the coil. The time of the phase-encode gradient was 0.5 ms, the field of view 32 mm, the recycle delay 1 s, and 64 averages were obtained per phase-encode step. Adiabatic pulses with a flip angle of 45° were used for uniform excitation. Total acquisition time was ~34 min. With this protocol, well-resolved spectra from 1-mm slices from the antero-septal region of the mouse heart parallel to the coil were obtained (8, 47). In a prior study (47) these noninvasive image-guided 31P MRS techniques gave identical results to those obtained from invasive measures, indicating minimal contamination from surrounding structures with this approach (47). All mice awoke within ~1 min after completing the MRI/MRS examination.

31P spectra were analyzed with a combination of custom (3) and proprietary (NIH Image, Bethesda, MD) software. The PCr/ATP ratio was determined from the integrated peak areas of the PCr and [γ-31P]ATP resonances from voxels centered on skeletal muscle in the anterior chest or on cardiac muscle identified from the high-resolution 1H MR images, as described previously (8). Voxel shifting was performed when necessary to optimize slice alignment with cardiac structures and to minimize skeletal muscle contamination of cardiac spectra (5). The PCr/ATP values were corrected for partial saturation effects using a factor determined in separate studies (6, 8, 46, 47) that included fully relaxed acquisitions. Infarcted, nonviable myocardium lacks PCr and ATP (45, 48). In prior 31P MRS studies (16, 18, 19, 32, 33, 37) of infarcted rodent hearts, the detected PCr and ATP signals were attributed to the surviving viable regions, even when the entire infarcted region was contained within the region studied by MRS. Based on this accepted practice and our efforts through animal positioning and slice selection to minimize infarcted tissue within the volume of interest, the cardiac PCr/ATP values reported here derive almost entirely from surviving, viable myocardium. Data were compared by ANOVA with STATISTICA software (StatSoft, Tulsa, OK). Differences were considered statistically significant at P < 0.05. All data means ± SD.

RESULTS

Postinfarction remodeling in mice. The mean body weight for all animals was 29 ± 2 g, and there were no statistically significant differences among the groups. There were no significant anatomic, functional, or metabolic differences between allopurinol- and oxypurinol-treated hearts, so the groups were combined (MI+XO1) (Table 1).

Representative 1H MR cardiac images acquired at end systole and end diastole are shown in Fig. 1 for a normal mouse (control) and in another after MI. Four weeks after MI, there was a significant increase in mean LV mass, a severefold increase in LV chamber dimensions, and a significant reduction in EF, as shown in Table 1. Specifically, LV end-diastolic and end-systolic dimensions were increased by more than sixfold, and mean LV ejection fraction decreased from ~60% to 15% (Table 1). After infarction, LV mass doubled (P < 0.001) and the ratio of myocardial mass-to-chamber volume was reduced severalfold (P < 0.001, Table 1), consistent with prior observations (36, 38) in infarct-remodeled hearts. Together, these findings in MI mice demonstrate a marked degree of geometric remodeling and LV dysfunction that occurs in this model of permanent left main coronary artery occlusion.

To determine whether myocardium remote from infarction demonstrates energetic abnormalities in the mouse similar to those observed in larger animals, we used noninvasive image-guided 31P MRS to quantify cardiac high-energy phosphates. Representative in vivo cardiac 31P MR spectra from normal and infarct animals are shown in Fig. 2. In control mice, the mean PCr/ATP ratio was 3.0 ± 0.6 in chest skeletal muscle and 2.1 ± 0.5 in heart. These agree well with previously published values in mice (8, 47), as well as those in larger species, including...
was significantly higher in MI
allopurinol and oxypurinol, respectively. The cardiac PCr/ATP
EF was significantly higher in all MI

tion normalized myocardial PCr/ATP (Fig. 3). The mean myo-
and 49
than in MI hearts (14
humans (6, 26, 46). In contrast, infarct remodeled myocardium
is characterized by a significant 30% decrease in the mean
cardiac PCr/ATP to 1.4 ± 0.6 (P < 0.02).
Effects of XOs in remodeled mouse myocardium. XOI
therapy did not affect the increase in LV mass that develops
after infarction but did significantly attenuate the marked
degree of ventricular dilatation that occurs (Table 1). Specifi-
cally, XOs attenuated the dramatic increase in ESV (P = 0.02)
and EDV (P = 0.03) after infarction (Table 1). In addition, LV
EF was significantly higher in all MI+XOI hearts (23 ± 9%) than
in MI hearts (14 ± 9%, P = 0.01). Infarct size did not
significantly differ between control and XO1 hearts (55 ± 11%
and 49 ± 9%, respectively; P = 0.10, Table 1), in accord with
prior histopathological findings in this model (43).
XO inhibition with allopurinol and oxypurinol after infarct-
normalized myocardial PCr/ATP (Fig. 3). The mean myo-
cardial PCr-to-ATP ratios were 2.1 ± 0.7 and 1.9 ± 0.4 for
allopurinol and oxypurinol, respectively. The cardiac PCr/ATP
was significantly higher in MI+XOI (2.0 ± 0.5) than in MI
mice (1.4 ± 0.6, P < 0.04) and similar to that in normal,
onfibracted mice.

There was a correlation between the metabolic and func-
tional parameters in MI and MI+XOI hearts (Fig. 4) in that the
correlation coefficient between PCr/ATP and ESV was −0.7
(P < 0.05) and between PCr/ATP and EF, r = 0.65 (P < 0.05).
Thus XO inhibition normalizes the reduced cardiac PCr/ATP
in these failing, infarct-remodeled mouse hearts, and this is
associated with improved contractile function.

DISCUSSION

The geometric, functional, and energetic consequences of
post-MI remodeling were noninvasively characterized in a
murine model of heart failure as well as the effects of XOs on
that process. We conclude that significant ventricular geometric
remodeling occurs 4 wk after permanent coronary ligation
in the mouse as evidenced by a doubling in LV mass, sever-
fold increases in EDV and ESV, as well as a marked
reduction in LV EF (Table 1). The magnitude of these changes
is comparable or larger than observed in other species (36).
Less dramatic changes in mass, chamber dimensions, and EF
have been reported in mice after reperfused infarction (50) and

![Figure 1](http://ajpheart.physiology.org/)

**A** Typical transverse short-axis 1H magnetic reso-
nance (MR) images of mouse thorax at level of heart at end
systole (A and B, left) and end diastole (A and B, right) for
normal mouse (control, A) and another 4 wk after MI (B).
After infarction there is severalfold increase in left ventricu-
lar (LV) dimensions and relative decrease in amount of
blood ejected. As a side note, blood in ventricular chamber
appears dark in normal mouse because blood with excited
spins has exited chamber during time of spin-echo se-
quence. In contrast, blood in ventricular chamber remains
bright in postmyocardial infarction (post-MI) heart with
same spin-echo sequence because only small fraction of
chamber blood with excited spins has exited chamber. RV,
right ventricle.

**B**
earlier than 4 wk after permanent coronary ligation (13). Less remodeling was also observed in other studies (30) where smaller infarct sizes (18 ± 2%) were induced than in this study (~50%), likely due to more proximal coronary ligation in our approach. The decrease in the ratio of LV mass to chamber volume is similar to that reported in other models with large infarctions after coronary occlusion (36, 38).

In addition, we observed a significant 30% reduction in the in vivo myocardial PCr/ATP in infarcted hearts. These data demonstrate that metabolic remodeling, at least in relation to the CK reaction, occurs in vivo in the postinfarction, failing mouse, and they are in agreement with other models with large infarctions after coronary occlusion (36, 38).

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Fig. 2. MR images (left) and 31P spectra (right) in normal (right, top), infarct-remodeled (right, middle), and infarct-remodeled with xanthine oxidase (XO) inhibitor (XOI) allopurinol (right, bottom) mouse hearts. 31P MR spectra with 1-dimensional chemical shift imaging were nominally obtained from 1-mm slices from antero-septal region of mouse heart parallel to coil (left). Cardiac phosphocreatine (PCr)-to-ATP ratio (PCr/ATP) is reduced in failing myocardium and normalized with chronic allopurinol administration. PPM, parts per million.

Marbán’s laboratory (43) recently showed that XO activity is increased in this mouse infarction model and that oral allopurinol suppresses this increase and improves both in vitro and in vivo contractile function as well as survival. The current MRI findings in different animals confirm the prior observation made with echocardiography that XO inhibition improves in vivo contractile function after infarction in the mouse and does so without preventing hypertrophy. In the earlier work, the improved contractile function with XOI was not associated with increased activator calcium or a left shift in calcium sensitivity but rather was due to an increase in force production during maximal calcium activation (43). Because allopurinol restored myofilament force generation to near-normal values without altering intracellular [Ca2+], the hypothesis was generated that XO inhibition improves the poor coupling between energy production and mechanics in failing hearts. Simply put, the ability to generate more force without augmenting activator calcium predicts an improved efficiency of myocardial energy utilization.

Energy metabolism fuels normal myocardial contractile function, and for decades it has been hypothesized that a deficit in energy metabolism may contribute to the contractile deficit in heart failure (21, 22). Likewise, a deficit in energy metabolism could contribute to the progressive dysfunction and geometric changes after infarction (29). The CK reaction reversibly converts the major cardiac form of chemical energy ATP with the prime energy reserve metab-
olite PCr. Animal models of heart failure and patients with heart failure typically exhibit abnormalities in the CK reactants with modest reductions in [ATP], larger reductions in [PCr] and total creatine, and significant reductions in the cardiac PCr/ATP (4, 10, 15, 16, 29, 34). Abnormalities, such as a reduced PCr/ATP, correlate with the severity of the heart failure, improve with clinical recovery, and are stronger predictors of mortality than usual clinical indexes of LV EF and the New York Heart Association class (31). All of these observations are consistent with but do not prove that the abnormalities in energy metabolism may contribute to the pathophysiology of the contractile dysfunction in heart failure. To test the energy starvation hypothesis of heart failure, one needs to determine whether a metabolic intervention that improves energetics results in improved contractile function in failing hearts. Evidence that overexpression of a glucose transporter in pressure-overload mice attenuates the development of heart failure (24) and that ranolizine, a free fatty-acid inhibitor, improves mechanical efficiency in dogs with heart failure (9) both indicate that metabolic interventions can be important in heart failure. However, here the energetic changes may be secondary to improved excitation-contraction coupling, rather than reflecting a primary metabolic effect.

In this regard, the current studies on XO inhibition in remodeled mouse myocardium provide important insights. XO inhibition improves mechanoeenergetic coupling in failing hearts by reducing energetic demand in both animals and people (7, 11). Improved mechanoeenergetic efficiency with XO inhibition occurs in failing but not normal hearts and is due to a reduction in myocardial oxygen consumption while maintaining or improving contractile function. Thus XO inhibition represents one strategy for evaluating the energy starvation hypothesis of heart failure.

We report here that XO inhibition normalizes the reduced myocardial PCr/ATP in failing mouse hearts. The improve-

Fig. 3. In vivo PCr/ATP values in chest muscle (left) and cardiac muscle (right) from control (white bars, n = 10), MI (black bars, n = 9), MI+XOIs (gray bars, n = 10) mice. *P < 0.02, statistically significant difference with control group (normal mice); #P < 0.02, statistically significant difference with MI group.

Fig. 4. Relationship between end-systolic volume [ESV, A, where ESV = −130.63 (PCr/ATP) + 354.64; r = −0.7], end-diastolic volume [EDV, B, where EDV = −133.76 (PCr/ATP) + 384.2; r = −0.7], and ejection fraction [EF, C, where EF = 12.761 (PCr/ATP) − 0.821; r = 0.65] and myocardial PCr/ATP in MI (○) and MI+XOI (■) hearts.
ischemia as a result of an imbalance of oxygen supply and demand whereby PCR is consumed to buffer or delay a decline in ATP. In the more chronic setting of heart failure, there is evidence that classic ischemia is not present in that deoxymyoglobin cannot be detected in several animal models of heart failure (2, 28). However, a slow loss in ATP does occur in heart failure that is accompanied by a more rapid and greater loss of total creatine (42). Creatine depletion acts to attenuate or prevent an increase in ADP as ATP falls (42). Because creatine is not synthesized in muscle cells, if expression of the major creatine transport proteins in animal models and patients with heart failure is depressed (35), then this may be the likely mechanism for the decrease in total creatine in heart failure. It seems likely that by improving mechanoenergetic coupling in dysfunctional myocardium and/or blocking adenine nucleotide degradation, XO inhibition attenuates the initial ATP loss and the resultant more dramatic decline in PCR/ATP. An alternative explanation posits that XOIs fundamentally alter cross bridge kinetics, such that more force is generated per ATP consumed. Such a mechanism has been proposed to underlie the effects of agents, such as XOIs, that increase maximal calcium-activated force without shifting the calcium-force relationship (39). We cannot exclude the possibility that XO inhibition exerts an antioxidant protective effect in the heart. The present data do not distinguish among these various mechanisms, which merit further dissection.

In conclusion, geometric, functional, and metabolic remodeling occurs in this mouse postinfarction model, and the magnitude of the changes is similar or greater than those observed in other larger mammals. XO inhibition attenuates but does not prevent the geometric changes, significantly improves contractile function, and completely normalizes depressed cardiac high-energy phosphate ratios. Because XO inhibition improves depressed energetics in postinfarction heart failure, additional studies of XO inhibition in other models of heart failure are warranted. The observation that a metabolic intervention normalizes energetics and results in improved contractile function directly supports the long-debated energy starvation hypothesis of heart failure. The widespread clinical availability of XO inhibitors should speed the spread clinical availability of these agents, such as XOIs, that increase maximal calcium-activated force without shifting the calcium-force relationship (39). We cannot distinguish among these various mechanisms, which merit further dissection.

ACKNOWLEDGMENTS

We thank Michelle Leppo for performing MI surgery on the mice. Present address: A. Naumova: University of Washington, 815 Mercer St., Office 131A, Seattle, WA 98109; Present name and address of L. Stull: Linda Marbán, Exicen, Inc., 2415 Old Bosley Rd., Lutherville, MD 21093.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-63030 (to R. G. Weiss) and ROI-HL-44065 (to E. Marbán).

DISCLOSURES

E. Marbán holds the Michel Mirowski, MD Professorship of Cardiology of the Johns Hopkins University. Under a licensing agreement between Cardiome Pharma Corporation and the Johns Hopkins University, E. Marbán is entitled to a share of royalty received by the University on sales of products described in this article. E. Marbán and the University own Cardiome Pharma Corporation stock, which is subject to certain restrictions under University policy. E. Marbán is a paid member of the Cardiome Pharma Corporation Scientific Advisory Board. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

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