Published in final edited form as:

J Cardiovasc Transl Res. 2011 August ; 4(4): 504–513. doi:10.1007/s12265-011-9276-0.

Effect of Acute Xanthine Oxidase Inhibition on Myocardial Energetics During Basal and Very High Cardiac Workstates

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Abstract

Myocardial ischemia is associated with reduced myocardial adenosine triphosphate (ATP) and increased free adenosine diphosphate (ADP) similar to the normal heart at very high cardiac workstates (HCW). We examined whether acute xanthine oxidase inhibition (XOI) in vivo can decrease myocardial free ADP in normal hearts functioning at basal cardiac workstates (BCW) or very HCW (catecholamine-induced). Myocardial high-energy phosphate (^{31}P magnetic resonance spectroscopy), blood flow (radioactive microspheres), and oxygen consumption (MVO_2) were measured in an open-chest canine model before and after infusion of vehicle or an XO inhibitor (allopurinol or febuxostat; $n = 10$ in each group) during BCW and infusion of dobutamine + dopamine to induce a very HCW. During BCW, both allopurinol and febuxostat resulted in higher phosphocreatine (PCr)/ATP, corresponding to lower ADP levels. During vehicle infusion, HCW caused a decrease of PCr/ATP and an increase in myocardial free ADP. Although XOI did not prevent an increase in free ADP during catecholamine infusion, the values in the allopurinol or febuxostat groups (0.141 ± 0.012 and $0.136 \pm 0.011 \mu\text{mol/g dry wt}$, respectively) remained significantly less than in the vehicle group (0.180 ± 0.017 ; $P < 0.05$). Thus, at a given rate of ATP synthesis, XOI decreased the free ADP level needed to drive ATP synthesis, suggesting a more energy-efficient status. As contractile dysfunction in ischemia is characterized by increase of myocardial free ADP and energy deficiency, the data suggest that XOI might be a potential therapy for improving energy efficiency during myocardial ischemia.

Keywords

Myocardial high-energy phosphates; Reactive oxygen species; Magnetic resonance spectroscopy; Allopurinol; Febuxostat

Introduction

Myocardial ischemia is associated with reduced myocardial adenosine triphosphate (ATP) due to progressive depletion of the total adenine nucleotide (TAN) pool, as well as increased myocardial free adenosine diphosphate (ADP) [1]. In in vivo normal canine hearts, myocardial high-energy phosphate (HEP) levels do not change during moderate increases of cardiac workload produced by catecholamine infusion to produce rate-pressure products (RPP) below 35,000 mmHg beats/min [1–3]. However, more intense catecholamine stimulation to very high work-states with RPP above 45,000 mmHg beats/min causes a decrease of the phosphocreatine (PCr)/ATP ratio reflecting an increase of cytosolic free ADP [1]. The decrease of myocardial PCr/ATP during very high workstates (HCW) implies that mitochondrial oxidative phosphorylation (OXPHOS) requires an increased level of free ADP to maintain the driving force for ATP production [1]. An increase of free ADP can activate adenylate kinase (myokinase) to catalyze the transfer of a phosphoryl group between two molecules of ADP to form one AMP and one ATP [4–6]. The increased AMP augments the conversion of AMP to adenosine. Adenosine can be further degraded via the purine degradation pathway to inosine, hypoxanthine, xanthine, and uric acid, which can be eliminated from the heart via myocardial perfusion [7]. Since the final two irreversible steps in the purine degradation pathway are catalyzed by xanthine oxidase (XO), it is possible that XO inhibition (XOI) might combat TAN loss during very high cardiac workstates. In addition, excess reactive oxygen species (ROS) can exert regulatory effects on bioenergetic and contractile performance [8, 9] so that ROS produced by XO may participate in these regulatory processes [10].

Since the above considerations suggest that XO has the potential to influence OXPHOS in the heart, we examined whether acute XOI can alter HEP levels in normal myocardium during basal or very high cardiac workstates using ^{31}P magnetic resonance spectroscopy (MRS). Using an awake instrumented dog model of rapid-pacing-induced congestive heart failure (CHF), we previously examined eight dogs during baseline and at two levels of treadmill exercise with or without administration of febuxostat (Takeda Pharmaceuticals North America, Inc.). The terminal open-chest magnet study results of these dogs with CHF were compared to five normal dogs. The results of the treadmill exercise study indicated that febuxostat improves LV function in dogs with CHF. However, the myocardial blood flow, coronary venous oxygen partial pressure, and myocardial oxygen consumption were not compared in normal dogs with or without febuxostat at baseline or high cardiac workstates [11]. Using normal dogs, the present study aimed to examine the effects of the two XO inhibitors febuxostat and allopurinol on myocardial energetics at very high cardiac workstates induced by high doses of catecholamine. Febuxostat is a new XO inhibitor that was FDA-approved in February, 2009 for the treatment of hyperuricemia in patients with gout [12–14]. Its non-purine structure conveys high selectivity for XO and avoids the cross-inhibition that allopurinol has on other purine and pyrimidine metabolism enzymes such as purine nucleoside phosphorylase and orotidine-5'-monophosphate decarboxylase [13, 15]. Allopurinol is a purine analog XO inhibitor used for treatment of gout. The results obtained using these two agents suggest that XO is involved in bioenergetic regulation in the heart and that XOI may act to improve cardiac energy efficiency.

Materials and Methods

Studies were performed with 30 normal adult mongrel dogs weighing 18–22 kg. All procedures and protocols were approved by the University of Minnesota Animal Care Committee. The investigation conformed to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Resources [16].

Experimental Preparation

Dogs were anesthetized using alpha-chloralose (loading dose of 100 mg/kg, iv; maintenance dose of 20 mg/kg/h, iv), intubated, and mechanically ventilated with oxygen-supplemented air to maintain arterial blood gases within physiological range. Catheters were introduced for accessing the ascending aorta (Ao), inferior vena cava (IV), left ventricle (LV), left atrium (LA), and coronary sinus (CS). Catheters were 3.0 mm outer diameter polyvinyl chloride, flushed with heparinized normal saline to prevent clotting, and secured using silk sutures after placement. The Ao catheter was placed through the right femoral artery. Two IV catheters were placed through the right femoral vein. The heart was accessed via a left thoracotomy through the fourth intercostal space and suspended in a pericardial cradle. The LV catheter was introduced through the apical dimple. Both the LA and CS catheters were introduced through the right atrial appendage. To restore the heart to its normal position, the pericardial cradle was then released.

A single loop transmit/receive radiofrequency (RF) coil for MRS was sutured onto the LV wall segment supplied by the left anterior descending coronary artery as described in detail previously [17]. The animals were placed into a polyethylene-lined Lucite cradle warmed to 37°C by a heating pad, and the MRS coil leads were soldered to a balanced-tuned circuit external and perpendicular to the thoracotomy incision. The animal was then positioned within the magnet for ^{31}P spectral acquisition.

Physiologically normal arterial blood gases and pH were maintained by adjusting mechanical ventilation. Hemodynamic parameters (Ao and LV pressure) were monitored using pressure transducers leveled with the heart and logged using a multi-channel direct writing recorder (Coulbourn Instruments, Allentown, PA, USA).

Drug Preparation

The allopurinol solution (6 mg/ml) was prepared by dissolving 600 mg of allopurinol (Sigma-Aldrich, St. Louis, MO, USA) in 50 ml of 0.1 N NaOH followed by diluting the solution to a final volume of 100 ml with 0.05 M sodium phosphate buffer. The febuxostat solution (5 mg/ml; Teijin Limited; Yamaguchi, Japan) was prepared by suspending 500 mg febuxostat in 90 ml of 0.05 M sodium phosphate buffer, dissolving via dropwise addition of 0.5 N NaOH (aq) to ~7.50 pH and diluting with additional phosphate buffer for a final volume of 100 ml. The combined dobutamine and dopamine solution (DbDp; 600 µg each/ml) was made by diluting 7.5 ml of dopamine stock solution (40 mg/ml) and 24 ml of dobutamine stock solution (12.5 mg/ml) in 468.5 ml of normal saline for a total volume of 500 ml.

Study Protocol

The experimental protocol for each animal consisted of three consecutive experimental intervals: a control period of basal cardiac work (BCW) followed by intravenous infusion of XO inhibitor (or vehicle) and finally the combined infusions of XO inhibitor (or vehicle) and DbDp. After a physiological steady state was achieved during each condition, data were collected to measure hemodynamics, myocardial blood flow (MBF), and oxygen consumption (MVO_2) by radionuclide-labeled microspheres and ^{31}P spectra. Aortic and

coronary sinus blood specimens (3 ml each) were withdrawn anaerobically into iced syringes for determination of blood O₂ content. XO inhibition was then induced by infusing allopurinol (0.27 mg/kg/min; *n*=10) or febuxostat (loading dose of 0.22 mg/kg/min for 10 min followed by maintenance dose of 0.06 mg/kg/min; *n*=10). Control animals (*n*=10) were infused with vehicle (0.05 M sodium phosphate buffer) at 0.012 ml/kg/min. After 30 min of inhibitor or vehicle infusion to reach steady state, a second data set was collected and then infusion of DbDp (20 µg/kg/min each) was initiated while the XO inhibitor or vehicle infusion continued. This level of catecholamine stimulation induced a very HCW (RPP >35,000 mmHg beats/min). A third data set was collected after ~10 min of catecholamine infusion, at which time a new physiological steady state was achieved. The approximate cumulative doses for allopurinol and febuxostat given during the experimental period were 11 and 4 mg/kg, respectively. At the end of the experiment, myocardial tissue samples were prepared from each animal. In each treatment group, myocardial biopsies from the four animals that did not receive radionuclide-labeled microsphere injections (for the determination of myocardial blood flow) were processed for the determination of purine metabolites by high-performance liquid chromatography (HPLC), and the hearts from the other six animals, which did receive microsphere injections, were fixed in a 10% formalin solution.

Magnetic Resonance Spectroscopy

The MRS methods have been described in detail previously [3, 17]. Briefly, MRS was performed using a 40-cm horizontal bore 4.7-T magnet (Oxford Instruments, Oxfordshire, UK) interfaced to a Sun Microsystems workstation (Sun Microsystems, Santa Clara, CA, USA) running VNMR 6.1 software (Varian Associates, Palo Alto, CA, USA). A single loop transmit/receive RF coil was custom-made from a 28-mm-diameter copper wire loop with 33-pF twisted silver leads and a reference capillary containing 15 µl of 3M phosphonoacetic acid. The coil was tuned for ¹H and ³¹P MRS at Larmor frequencies of 80.3 and 198.3 MHz, respectively. The ¹H MRS signal of water was used for B₀ shimming, positioning the coil within the magnetic isocenter and determining the spatial coordinates for spectroscopic localization.

Hemodynamic steady-state values were achieved for each experimental condition before acquiring spectra and other data. Both spectra acquisition and respiratory ventilation were gated to the LV pressure, with acquisition triggered during late diastole and ventilation triggered between acquisitions. Transmural profiles of HEP, inorganic orthophosphate (*P*_i), and 2,3-diphosphoglycerate over 5 voxels were acquired using spatially localized ³¹P spectroscopy. The rotating-frame experiment using adiabatic plane-rotation pulses for phase modulation-imaging-selected in vivo spectroscopy method was used as previously described [17]. Briefly, this MRS method achieves spatial discrimination using the inherent spatial variation along the RF field produced by a single loop transmit/ receive RF coil. Spatial information was systemically encoded as harmonics of various orders in the MRS signal via stepwise lengthening of the RF excitation pulse.

A pulse repetition time of 6 to 7 s was used to permit full relaxation of ATP and *P*_i resonances and ~90% relaxation of PCr resonance. PCr resonance intensities were corrected for this minor saturation as previously described [18]. Chemical shifts were measured relative to PCr, which was assigned a chemical shift of -2.55 ppm. Resonance intensities were quantified using integration routines provided by VNMR software (Varian, Inc., Palo Alto, CA, USA). Spectral peaks were normalized to baseline. ATP was determined by ATP-γ resonance. *P*_i was measured relative to baseline values (Δ*P*_i) using integrals obtained in the *P*_i resonance region. PCr/ATP and Δ*P*_i/PCr resonance integral ratios were calculated.

Myocardial Perfusion and Oxygen Consumption

The distribution of MBF across the wall of the LV was estimated using 15- μ m-diameter microspheres labeled with the gamma-emitting radionuclides ^{141}Ce , ^{51}Cr , ^{95}Nb , ^{85}Sr , or ^{46}Sc (NEN, Boston, MA, USA) as described previously [17, 19]. Briefly, MBF measurements were obtained by injecting 2×10^5 microspheres through the left atrial catheter while a reference arterial blood specimen was withdrawn from the aortic catheter at a rate of 15 ml/min. At the conclusion of study, the heart was excised and fixed in 10% buffered formalin. The myocardium directly beneath the spectroscopy coil was cut into three transmural layers from epicardium to endocardium, weighed and placed into scintillation vials for counting of radioactivity and determination of blood flow. MVO_2 was calculated as difference in oxygen content between aortic and coronary vein blood multiplied by MBF.

Tissue Preparation

Immediately following the acquisition of the final ^{31}P MR spectrum, four drill biopsies (50 mg each) were taken from the anterior LV wall near the apex during the XO inhibitor + DbDp infusions and snap-frozen in liquid nitrogen. Biopsy specimens were stored in liquid nitrogen until HPLC analysis. Hearts used for myocardial perfusion and oxygen consumption determination were immediately fixed in a 10% solution of buffered formalin prior to analysis.

HPLC Analysis of Purine Metabolites

Tissue biopsies were extracted by homogenization in 0.42 M perchloric acid (10%, w/v). Following centrifugation, the supernatant was titrated with 1 M potassium hydroxide to pH 7.0–7.5 with phenol red as the pH indicator. The samples were then injected into HPLC for determination of creatine, PCr, and ATP levels.

Data Analysis

Hemodynamic data were taken from the chart recordings. MBF was determined from microsphere measurements. MVO_2 was calculated as the product of myocardial blood flow and the difference in O_2 content between aortic and anterior coronary vein blood samples. ^{31}P MRS spectra were analyzed as described above. Myocardial free ADP content was calculated using the creatine kinase equilibrium of $[\text{ADP}]_{\text{free}} = [\text{ATP}][\text{Cr}]_{\text{free}} / [\text{PCr}][\text{H} +]K_{\text{eq}}$, where $K_{\text{eq}} = 1.66 \times 10^9 \text{ M}^{-1}$, cardiomyocyte sarcoplasmic pH was determined to be 7.05, and $[\text{Cr}]_{\text{free}} = [\text{Cr}]_{\text{total HPLC}} - [(\text{PCr}/\text{ATP})_{\text{MRS}} \cdot \text{ATP}_{\text{HPLC}}]$. $[\text{Cr}]_{\text{total}}$ (sum of PCr and Cr values) and ATP were obtained from HPLC biopsy measurements, and PCr/ATP was derived from spatially localized myocardial ^{31}P MR spectra. PCr and ATP resonance integrals were normalized to those present at baseline to determine if values were changed by the experimental interventions.

Statistical Methods

The effects of infusions at BCW and HCW compared to baseline within each drug treatment group were analyzed using one-way repeated measures ANOVA with post hoc Bonferroni t tests at $P < 0.05$ for significance. Differences across drug treatment groups during each specific experimental interval were analyzed using one-way ANOVA with post hoc Bonferroni t tests at $P < 0.05$ for significance. All values are expressed as mean $\pm$ SE.

Results

Hemodynamic Data

Hemodynamic data are summarized in Table 1. Left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure, and aortic pressure (Ao) at baseline and during

infusion at a BCW were not significantly different across the vehicle, allopurinol, and febuxostat groups. At BCW, animals receiving allopurinol or febuxostat exhibited a modest significant increase in heart rate as compared to vehicle controls; however, these changes were not significantly different from their respective pre-infusion baseline. A similar response was observed for the RPP in the allopurinol treated animals. In response to catecholamine stimulation, RPP increased to greater than 45,000 mmHg beats/min in all groups as a result of significant increases in heart rate and LVSP. At HCW, RPP was slightly but significantly lower in febuxostat treated dogs versus vehicle controls due to a slightly lower LVSP in the febuxostat group.

Myocardial Perfusion and Oxygen Consumption

MBF and MVO₂ data are summarized in Table 2. Transmural MBF and MVO₂ at baseline and during drug infusion at BCW were not significantly different within or across the three treatment groups. Regional and mean MBF values increased during the HCW, but there were no significant differences between groups. MVO₂ during BCW tended to decrease with vehicle and tended to increase following allopurinol, but these changes were not significant. During the HCW, MVO₂ increased approximately two-fold from baseline in each treatment group, with no significant differences among the groups. No significant differences existed in the coronary arteriovenous O₂ difference or the subendocardial-to-subepicardial myocardial blood flow ratio within or across the groups during any of the three experimental conditions.

Myocardial High-Energy Phosphate Levels

Figure 1 illustrates typical transmural ³¹P MR spectra obtained from hearts in each of the three treatment groups. HEP data obtained from MRS are summarized in Table 3. In vehicle control animals, PCr/ATP decreased significantly at HCW (from 1.97±0.09 to 1.63±0.08, *P*<0.05). Both allopurinol and febuxostat caused significant increases in PCr/ATP when each drug was infused at BCW; PCr/ATP ratios also fell significantly after the onset of HCW in these two groups. However, because of the elevation of PCr/ATP at BCW during drug infusions, PCr/ATP values during HCW were not different from pre-infusion baseline values. For all treatment groups, myocardial Δ*P*_i/PCr did not change significantly between baseline and drug infusion at BCW but increased significantly at high workstates; there were no significant differences among the treatment groups.

Biopsy Data and Estimated Myocardial Free ADP Levels

Table 4 shows absolute concentrations (micromoles per gram dry weight myocardium) of myocardial ATP and total creatine (sum of PCr and free Cr values) analyzed by HPLC from biopsies obtained at the end of the catecholamine infusion period. PCr levels at all experimental intervals and ATP during baseline and infusion at a basal workstate were estimated using the ATP values measured by HPLC at HCW and the PCr/ATP ratios obtained from MRS. ATP levels did not differ between groups and did not change significantly in response to changes in cardiac work. Total creatine levels were similar among the three groups.

Myocardial free ADP content was calculated using the creatine kinase equilibrium as described previously [17]. In vehicle control animals, estimated free ADP increased significantly at HCW (0.180±0.017 vs. 0.127±0.012 μmol/g dry wt, *P*<0.05). Both allopurinol and febuxostat caused significant decreases in free ADP during BCW. In both of these groups, free ADP increased significantly at HCW. However, because both drugs reduced ADP levels at BCW, free ADP values at HCW were not different from the pre-infusion baseline values and were significantly lower than in the vehicle control group at

HCW (0.141 ± 0.011 and 0.136 ± 0.011 $\mu\text{mol/g}$ dry wt for allopurinol and febuxostat, respectively, vs. 0.180 ± 0.017 for vehicle).

Discussion

In this *in vivo* study of the acute bioenergetic effects of XO_i in normal hearts, we report three major findings. First, acute XO_i applied during basal workstate conditions resulted in significant increases of myocardial PCr/ATP values and significant decreases of calculated myocardial free ADP levels compared to those in untreated animals. Second, XO_i did not attenuate the decreases of myocardial PCr/ATP and increases of calculated free ADP that occurred during the high workstate induced by catecholamine stimulation. This indicates that factors independent of XO activation were responsible for the HEP changes that occurred during catecholamine stimulation. However, because XO_i caused increased PCr/ATP and decreased free ADP levels during basal workstate conditions, the values for PCr/ATP and free ADP during catecholamine infusion were similar to those during the basal workstate in untreated animals. Finally, the changes in PCr/ATP and free ADP were essentially identical whether XO_i was accomplished with allopurinol or with febuxostat, implying that inhibition of XO, rather than nonspecific effects of the drugs, was responsible for the observed changes.

In the present study, ATP and creatine were measured in myocardial biopsy specimens, and PCr/ATP determined with ^{31}P NMR spectroscopy was used to calculate cytosolic free ADP according to the creatine kinase (CK) equilibrium. Since the CK reaction is near equilibrium, acute increases in cytosolic free ADP result in reciprocal changes of PCr/ATP, provided that total cellular creatine remains constant and there are no marked changes in ATP content [20]. More than 90% of the ATP utilized by the heart is produced through oxidative phosphorylation in the mitochondria. In the *in vivo* heart, the increased ATP generation required to support moderate increases of cardiac work occur with no change in cytosolic free ADP [14, 21]. However, at very high workstates, as achieved during catecholamine infusion in the present study, ADP levels increase and are presumed to contribute to stimulation of the increased ATP production [2, 14]. Similar reductions of PCr/ATP have been observed in hearts of untrained human subjects and athletes during dobutamine/atropine stress testing [22, 23]. An increase in myocardial free ADP during catecholamine administration could be the result of insufficient oxygen availability during the high workstate. However, we previously excluded that possibility by the finding that myocardial myoglobin remained fully saturated with oxygen during the high workload [24]. Thus, the increase in ADP appears to be fundamental response that contributes to the increased ATP production during very high workstates in the normal heart.

Several investigators have previously reported the effects of acute XO_i on myocardial contractile function and oxygen consumption. Ukai et al. [25] administered allopurinol to awake dogs during basal conditions and during a modest increase in cardiac work produced by dobutamine ($8 \mu\text{g/kg/min}$ i.v.). Allopurinol did not alter heart rate, LV systolic pressure, or LV dP/dt_{max} either during basal conditions or in response to dobutamine in normal animals, although allopurinol did cause a small but significant increase in LV dP/dt_{max} during dobutamine stimulation in animals with pacing-induced heart failure. Ekelund et al. [26] reported that allopurinol caused no change in indices of contractility in normal dogs and no change in myocardial oxygen consumption, although allopurinol did cause an increase in contractility that was associated with a decrease of myocardial oxygen consumption in dogs with pacing-induced heart failure. In agreement with these previous reports, in the present study, neither allopurinol nor febuxostat caused significant changes in hemodynamics or in myocardial oxygen consumption in the normal canine heart.

Both allopurinol and febuxostat caused increases of PCr/ATP, which remained significantly higher than in the vehicle treated hearts even during the very high workload produced by catecholamine infusion. Since XO is unlikely to have caused a change in size of the total creatine content of the myocytes, the increase of PCr/ATP implies a decrease of free ADP. Such a decrease of ADP would result in an increased free energy of ATP hydrolysis. The mechanism by which XO caused this effect is not entirely clear but may have involved suppression of superoxide production by XO, since ROS at low concentrations are thought to participate in mtOXPHOS regulatory processes [10]. Myocardial ROS arise primarily from the electron transport chain [8, 27], but are also generated by XO [28], NADPH oxidase [21], and uncoupled NO synthase [29, 30]. ROS produced in the myocardium include superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the toxic hydroxyl radical ($OH^{\cdot-}$) [31]. Additionally, the interaction between $O_2^{\cdot-}$ and nitric oxide (NO) produces reactive nitrogen species such as peroxynitrite ($ONOO^-$) [32]. Oxidative ATP synthesis depends on the integrity of mitochondrial subsystems including the tricarboxylic acid cycle, the electron transport chain, and ATP synthase, several of which can be adversely affected by ROS-mediated stress [2, 33–35]. ROS can inhibit the mitochondrial F_1F_0 -ATPase with resultant slowing of respiratory chain activity that would require free ADP to rise to maintain OXPHOS rates [36]. ROS activate mitochondrial uncoupling proteins which cause increased proton leak and a reduction of the electrochemical gradient across the inner mitochondrial membrane [37, 38]. A reduction of the proton-motive force would increase the concentration of cytosolic ADP required to support a given rate of ATP synthetic activity by the F_0F_1 -ATPase, so a decrease of ROS generation after XO could have contributed to the decreased free ADP, which is consistent with the effects observed in this study.

Unlike a previous report in which we observed a significant decrease in myocardial ATP levels during intense catecholamine stimulation [18], in the present study, ATP values did not decrease significantly. However, in the previous study, the duration of catecholamine infusion was more than three times longer than in the present protocol. Since the loss of ATP during very high workloads occurs gradually, the relatively brief single catecholamine infusion used in the present study was insufficient to cause significant loss of ATP.

It was predicted that XO inhibition would be beneficial in heart failure given alterations in oxidative phosphorylation, and in animal studies, it was reported to improve contractile function and decrease MVO_2 in failing hearts [10, 36, 39]. However, data from OPT-CHF, a large, multicenter trial of XO inhibition in heart failure, showed no benefit of oxypurinol (600 mg daily) compared to placebo on clinical outcomes in unselected patients with moderate to severe LV dysfunction [40]. More recently, the effects of XO on endothelial function and myocardial ischemia have been evaluated. George et al. demonstrated improvement in endothelial function by reducing vascular oxidative stress in a randomized control trial [41]. Similarly, Noman et al reported improvement in exercise-induced myocardial ischemia in patients given high-dose allopurinol (600 mg daily) [42]. ATP preservation was postulated to be the mechanism for clinical improvement in ischemia [42]. Although we did not measure blood levels of the XO in the present study, in pharmacokinetic studies done prior to this research, we found that the febuxostat infusion protocol used in this study resulted in plasma drug concentrations in dogs of 5.8–8.7 $\mu\text{g/ml}$, which are within the range associated with a therapeutic effect in humans.

Limitations

One limitation of this study is the lack of direct measurements of ROS in myocardial biopsies or coronary venous blood. These measurements, which were beyond the scope of this study, will be needed in future studies of the effects of XO in normal and diseased hearts. Although these data will be crucial to further test the hypotheses generated by the

current observations, our working interpretation of the mechanisms of XOI effects is consistent with their well-described actions. The studies described in this study examined effects of XOI in normal hearts. Additional studies of the effects of XOI will be needed to examine the effects of XOI on bioenergetics in the ischemic heart.

Conclusions

During basal workstate conditions, administration of allopurinol or febuxostat resulted in lower cytosolic free ADP levels without altering the rate of ATP synthesis (as reflected by MVO₂ values). The finding that a lower level of ADP was required to drive a given rate of ATP synthesis after XOI suggests a more efficient myocardial energy status. However, XOI did not prevent the reduction of PCr/ATP and the increase of ADP that occurred in non-XOI inhibited hearts during very high workstates. The persistence of the catecholamine-induced changes of PCr/ATP and ADP after XOI implies that additional catecholamine-activated mechanisms affect the kinetics of OXPHOS. It may be that XO-mediated production of superoxide exerts a regulatory role on mtOXPHOS so that XOI results in more efficient ATP production. As the function of the ischemic heart is characterized by increased myocardial free ADP, these data suggest that XOI inhibition may have a potential for improving energetic efficiency during acute myocardial ischemia.

Acknowledgments

The authors gratefully acknowledge Drs. Lin Zhao and Jerry Wessale, Takeda Global Research & Development Center, Inc., Deerfield, IL, for their scientific contributions to the study. This work was supported by an American Heart Association Predoctoral Fellowship Award (0415468Z to J. Lee), National Institutes of Health Medical Scientist Training Program Grant (GM008244 to University of Minnesota Combined MD–PhD Program), and US Public Health Service Grants NIH RO1 HL50470 and HL67828 UO1HL 100407. This work was supported in part by a research grant awarded by TAP Pharmaceutical Products, Inc. (now a part of Takeda Global Research & Development Center, Inc., Deerfield, IL, USA)

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Fig. 1. Typical transmural ^{31}P MR spectra obtained from hearts in each of the three treatment groups

Table 1

Hemodynamic data

Treatment (n=10/group)	Experimental conditions	HR (beats/min)	LVSP (mmHg)	LVEDP (mmHg)	Mean Ao (mmHg)	RPP (10 ³ mmHg beats/min)
Vehicle	Baseline	119±5	110±5	3.9±0.3	79±6	13.17±0.89
	Infusion @ BCW	114±8	115±6	4.1±0.4	87±6	13.24±1.33
	Infusion @ HCW	181±8*	288±13*	9.2±0.7	115±12*	51.82±2.37*
Allopurinol	Baseline	138±7	123±5	4.2±0.7	92±6	17.16±1.41
	Infusion @ BCW	152±7**	134±9	5.2±0.6	91±4	20.69±2.10**
	Infusion @ HCW	196±7*	269±15*	7.9±0.8	110±13	52.66±3.24*
Febuxostat	Baseline	141±6	122±5	4.8±0.7	93±4	17.34±1.08
	Infusion @ BCW	150±8**	117±5	4.7±0.6	86±5	17.73±1.47
	Infusion @ HCW	186±7*	249±12*,***	8.9±1.0	118±9*	45.92±2.22*,***

Values are mean±SE

n number of dogs, HR heart rate, LVSP left ventricular systolic pressure, LVEDP left ventricular end-diastolic pressure, Mean Ao mean aortic pressure, RPP rate-pressure product, BCW basal cardiac workstate, HCW high cardiac workstate

* P<0.05 vs baseline and infusion @ BCW;

** P<0.05 vs vehicle group

Table 2

MBF and MVO₂ data

Treatment	Experimental conditions (n=5-6/group)	Subendo (ml/min/g; wet wt)	Mid (ml/min/g; wet wt)	Subepi (ml/min/g; wet wt)	Mean (ml/min/g; wet wt)	Subendo/subepi	a-v (ml O ₂ /100 ml; n=3-6)	MVO ₂ (ml/min/100 g; wet wt, n=3-6)
Vehicle	Baseline	0.83±0.16	0.76±0.15	0.70±0.14	0.76±0.15	1.19±0.10	11.3±0.7	10.4±2.0
	Infusion @ BCW	0.70±0.05	0.70±0.08	0.72±0.09	0.71±0.07	0.99±0.05	10.3±0.5	7.7±1.3
	Infusion @ HCW	1.36±0.18*	1.41±0.17*	1.44±0.17*	1.40±0.17*	0.94±0.05	11.8±0.2	17.8±2.8*
Allopurinol	Baseline	0.88±0.15	0.85±0.14	0.83±0.15	0.85±0.15	1.08±0.04	11.0±1.1	7.9±1.6
	Infusion @ BCW	1.01±0.11	0.96±0.10	1.06±0.13	1.01±0.11	0.96±0.05	10.8±0.4	11.7±1.2
	Infusion @ HCW	1.87±0.38*	1.77±0.32*	1.74±0.27*	1.79±0.32*	1.05±0.09	11.4±0.4	19.0±5.2
Febuxostat	Baseline	0.77±0.07	0.76±0.07	0.67±0.05	0.73±0.06	1.15±0.05	11.6±0.4	8.5±0.7
	Infusion @ BCW	0.75±0.13	0.69±0.09	0.70±0.08	0.71±0.09	1.07±0.12	10.7±0.9	7.9±1.8
	Infusion @ HCW	1.57±0.32*	1.51±0.25*	1.39±0.20*	1.49±0.25*	1.12±0.13	10.7±0.8	17.6±2.6*

Values are mean±SE

n number of dogs, *Subendo* subendocardial myocardial blood flow, *Mid* midmyocardial blood flow, *Subepi* subepicardial myocardial blood flow, *Mean* average myocardial blood flow, *a-v* the difference in aortocoronary sinus O₂ content, *MVO₂* myocardial O₂ consumption, *BCW* basal cardiac workstate, *HCW* high cardiac workstate, *wt* weight

* *P*<0.05 vs baseline and infusion @ BCW

Table 3

HEP data obtained from MRS

Treatment (n=8-10/group)	Experimental conditions	$\Delta P_i/P_{Cr}$	PCr/ATP
Vehicle	Baseline	nd	1.97±0.09
	Infusion @ BCW	0.02±0.02	1.99±0.09
	Infusion @ HCW	0.13±0.04*	1.63±0.08*
Allopurinol	Baseline	nd	2.04±0.08
	Infusion @ BCW	-0.005±0.02	2.36±0.12***
	Infusion @ HCW	0.11±0.02*	2.08±0.11**
Febuxostat	Baseline	nd	2.05±0.07
	Infusion @ BCW	0.02±0.02	2.24±0.06***
	Infusion @ HCW	0.16±0.03*	2.04±0.10***

Values are mean±SE

n number of dogs, P_i inorganic phosphate, P_{Cr} phosphocreatine, ATP adenosine triphosphate, BCW basal cardiac workstate, HCW high cardiac workstate, nd not detectable

* $P<0.05$ vs baseline;

** $P<0.05$ vs vehicle group

Table 4

Absolute concentrations (micromoles per gram dry weight myocardium) of myocardial ATP and total creatine (sum of PCr and free Cr values)

Treatment	Experimental conditions	Cr _{total} (n=4)	ATP (n=4)	PCr, calculated (n=8-10)	ADP _{free} , calculated (n=8-10)
Vehicle	Baseline	nd	16.3±1.7 ^a	32.1±1.5	0.127±0.012
	Infusion @ BCW	nd	15.3±1.6 ^a	30.4±1.3	0.132±0.012
	Infusion @ HCW	68.1±8.8	16.0±1.7	26.1±1.4 ^{*,***}	0.180±0.017 ^{*,***}
Allopurinol	Baseline	nd	17.6±2.3 ^a	35.9±1.3	0.138±0.009
	Infusion @ BCW	nd	16.4±2.1 ^a	38.7±1.9 ^{**}	0.116±0.012 [*]
	Infusion @ HCW	77.0±9.6	16.8±2.2	35.0±1.8 ^{***,****}	0.141±0.011 ^{***,****}
Febuxostat	Baseline	nd	18.6±2.4 ^a	38.2±1.3 ^{***}	0.118±0.009
	Infusion @ BCW	nd	18.0±2.3 ^a	40.5±1.2 ^{***}	0.099±0.006 ^{*,***}
	Infusion @ HCW	73.0±7.4	16.7±2.1	33.9±1.6 ^{***,****}	0.136±0.011 ^{***,****}

Values (micromoles per gram, dry weight) are mean±SE

n number of dogs, Cr_{total} total myocardial creatine, ATP adenosine triphosphate, ADP adenosine diphosphate, PCr phosphocreatine, BCW basal cardiac workstate, HCW high cardiac workstate, nd not determined

* P<0.05 vs baseline;

** P<0.05 vs vehicle group;

*** P<0.05 vs infusion @ BCW

^a Calculated value