Allopurinol Improves Cardiac Dysfunction After Ischemia-Reperfusion via Reduction of Oxidative Stress in Isolated Perfused Rat Hearts

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It has been reported that the xanthine oxidase inhibitor, allopurinol, has a protective effect on ischemia–reperfusion injury, but the precise mechanism of its action is still unclear. Therefore, in the present study the mechanisms of the myocardial protection of allopurinol were evaluated in isolated perfused rat hearts. Allopurinol significantly inhibited myocardial xanthine oxidase activity, and improved left ventricular dysfunction after ischemia–reperfusion. In addition, the lactate dehydrogenase content in the coronary effluent obtained after reperfusion was significantly decreased. ATP, ADP, AMP and IMP significantly decreased, whereas inosine, hypoxanthine and xanthine significantly increased after ischemia in both the control and allopurinol groups. The concentration of xanthine was significantly decreased after ischemia–reperfusion in the allopurinol group; however, allopurinol did not affect the other purine metabolites. To evaluate the accumulation of oxidative stress, thiobarbituric acid reactive substances (TBARS) production in myocardial tissue was measured and allopurinol significantly decreased TBARS formation after ischemia–reperfusion. Finally, myocardial hydroxyl radicals were directly measured by electron spin resonance spectroscopy with the nitroxide radical 4-hydroxy-2, 2,6,6-tetramethylpiperidine-N-oxyl. Hydroxyl radicals significantly increased immediately after reperfusion, but were significantly decreased in the allopurinol group. In conclusion, allopurinol reduced myocardial injury after ischemia–reperfusion by suppressing oxidative stress, but not by salvage of ATP. These findings may lead to the development of new therapeutic strategies for myocardial ischemia–reperfusion injury. (*Circ J* 2003; 67: 781–787)

Key Words: Ischemia; Myocardial infarction; Oxygen; Reperfusion

It is well known that ischemia–reperfusion injury is mediated by the production of free radicals in tissues such as the heart, lungs, kidneys, intestine, and brain.^{1,2} In recent years, coronary reperfusion therapy has become a common therapeutic strategy for patients with acute myocardial infarction, but fatal arrhythmia and myocardial dysfunction, known as reperfusion injury, frequently occur immediately after coronary reperfusion and sometimes leads to critical complications. Thus, it is of clinical importance to develop novel therapeutic strategies for ischemia–reperfusion injury.

It has been reported that the xanthine oxidase inhibitor, allopurinol, and its active metabolite, oxypurinol, have a protective effect against ischemia–reperfusion injury in various species: dogs,^{3,4} rats,^{5,6} rabbits^{7,8} and humans.^{9,10} The reported effects have been a reduction in the size of the infarction,³ improvement of ventricular function^{4,7} a lower incidence of arrhythmias^{5,8} and decreased release of myocardial creatine kinase⁶ However, the precise mechanism

of the protective effect of allopurinol in the ischemic myocardium is still unclear. One possibility is inhibition of free radicals. The conversion of hypoxanthine and xanthine via xanthine oxidase is the major potential source of free radicals, which have been proposed as playing an important role in the genesis of ischemia–reperfusion injury.^{3,4,11} However, it is still controversial whether xanthine oxidase inhibitors improve myocardial injury via a reduction of oxidative stress, because direct measurement of myocardial xanthine oxidase-mediated free radical generation has not been performed.

In the present study, therefore, we directly measured myocardial hydroxyl radicals by electron spin resonance (ESR) spectroscopy using for the first time the nitroxide radical 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (hydroxy-TEMPO).

Methods

Isolated Heart Preparation

Male Wistar rats (300–350 g, 12 weeks old) were used for the isolated isovolumic heart preparations. All animals were handled in strict accordance with the Tottori University Guide for the Care and Use of Laboratory Animals. Isolated perfused rat hearts were created as previously described.¹² In brief, after the rats were heavily anaesthetized by intraperitoneal injection of a cocktail of ketamine HCl (40 mg, Sigma, St Louis, MO, USA) and xylazine (2.0 mg, Sigma) dissolved in heparin (1,000 IU, Shimizu Seiyaku,

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Shimizu, Japan), the heart was rapidly excised, and the aorta was cannulated for retrograde perfusion with a 14-gauge needle connected to a modified Langendorff perfusion system, which consisted of a warmed storage vat and a condenser at 37°C, and an adjustable-speed rotary pump (model 7523-40, Masterflex, Barrington, IL, USA). Throughout the experiment, the coronary perfusion pressure (CPP) was held at 80 mmHg by changing the coronary perfusion flow of modified Tyrode's solution (in mmol/L: NaCl 144, KCl 5, CaCl₂ 1.5, MgCl₂ 0.9, N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulphonic acid] (HEPES) 6, and glucose 5, pH 7.4) equilibrated with 100% oxygen. The perfusate was not recirculated. Ventricular function was assessed by measuring the left ventricular pressure (LVP) with a fluid-filled latex balloon inserted into the left ventricle through the mitral valves and inflated to give an end-diastolic pressure of 5 mmHg. The transducer was connected to a Mac Lab System (model Power Lab 8sp, AD Instruments, Castle Hill, NSW, Australia) and the LVP and CPP were measured. After being attached to the Langendorff perfusion system, the heart was allowed to stabilize for 30 min, during which time LVP and CPP (constant CPP at 80 mmHg) were monitored.

Experimental Protocol

All hearts were perfused for 30 min to allow stabilization of LVP and CPP, and then subjected to 25 min of global ischemia, followed by 30 min of reperfusion. In the allopurinol group, the hearts were perfused with allopurinol (1.0 mmol/L, Sigma) dissolved directly in the Tyrode's solution for 10 min before ischemia, and continuously for 30 min of reperfusion. During the ischemic period, the hearts were placed in Tyrode's solution in a water-jacketed container to maintain the temperature constant at 37°C. We measured preischemic and postischemic values for LVP, CPP and coronary perfusion flow throughout the experiment. To eliminate the effect of changes in heart rate, pacing was performed at a constant rate (240 beats/min) before ischemia and after reperfusion using a pacing generator (model SEN3201, Nihon Kohden, Tokyo, Japan) as previously described.¹³ Myocardial tissue was collected immediately before global ischemia, immediately before reperfusion and at 1, 5 and 30 min after reperfusion. The samples were quickly frozen in liquid nitrogen and stored at -80°C until assays for the measurement of purine metabolites and reactive oxygen species (ROS).

Measurement of Lactate Dehydrogenase (LDH) Activity

To analyze the release of LDH, coronary effluent was also collected for 1 min before and after reperfusion. LDH activity was determined using an LDH assay kit (cicaliquid LDH, Kantokagaku, Tokyo, Japan). The absorbance was measured at 340 nm. LDH activity was expressed as IU·min⁻¹·g⁻¹ wet tissue weight.

Measurement of Xanthine Oxidase Activity

Xanthine oxidase activity was measured as previously described.^{14,15} Briefly, fresh myocardial tissue was homogenized in ice-cold 5 mmol/L phosphate buffer (pH 7.4) containing 1.0 mmol/L EDTA, 1.0 mmol/L dithiothreitol and 0.1 mmol/L phenyl-methyl sulfonyl fluoride. The mixture was centrifuged at 7,700 G for 30 min at 4°C (Model RM150, Tomyseiko, Tokyo, Japan). After preincubation for 5 min at 37°C of 240 µl 0.2 mol/L Tris-HCl buffer (pH 9.0) containing 113 µmol/L pterin with or without 4 mmol/L

NAD, the enzyme reaction was started by adding 60 µl of the supernatant. After incubation for 10 to 30 min, 100 µl of the reaction mixture were removed and added to 1.5-ml tubes containing 100 µl of 4% HClO₄. The resulting mixture was vigorously shaken with an agitator and then centrifuged at 15,000 G. A portion (150 µl) of the supernatant was neutralized with 6 µl of 5 mol/L K₂CO₃, and 20 µl of the neutralized supernatant were used to measure xanthine oxidase activity by high-performance liquid chromatography (HPLC) (LC-6A HPLC apparatus, RF 530 fluorescence HPLC monitor, C-R3A chromatopac recorder, Shimadzu, Kyoto, Japan). Xanthine oxidase activity was expressed as nmol·g protein⁻¹·h⁻¹.

Measurement of Adenine Nucleotides and Purine Metabolites in Myocardial Tissue

Adenine nucleotides and purine metabolites were measured as previously described.¹⁶ Frozen myocardial tissue was homogenized (10% wt/vol) in 6% trichloroacetic acid (TCA) and centrifuged at 17,000 G for 10 min at 4°C (MX-180 centrifuge, Tomyseiko, Tokyo, Japan). The supernatant was then removed and added to an equal volume of the tri-*n*-octylamine in freon (Sigma), vortex-mixed for 2 min, and centrifuged at 700 G for 3 min. The aqueous layer was filtered through a 0.22-µm Millipore filter. The amount of purine in myocardial tissue was determined by HPLC (L6000 pump, L4000 UV Detector, HITACHI, Tokyo, Japan). To separate nucleotides [adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP)], we used a mobile phase buffer containing 10 mmol/L NaH₂PO₄, 5 mmol/L tetrabutyl-ammonium-bromide, and acetonitrile, adjusted to pH 6.0. Nucleotides (inosine, hypoxanthine, xanthine) were then separated using the buffer, 60 mmol/L KH₂PO₄, 0.45 mmol/L tetra-*n*-butylammonium phosphate and 0.35 mol/L acetonitrile, adjusted to pH 3.0. All buffers were filtered through 0.45-µm Millipore filters. Nucleotides and nucleosides were identified by comparing their retention time with those of standard samples [ATP, ADP, AMP, IMP, inosine, hypoxanthine, xanthine (Sigma)], and we determined the concentration of each sample on the basis of the peak area using external standards. The amount of purine metabolites in myocardium was expressed as µmol/g wet tissue weight.

Measurement of Thiobarbituric Acid Reactive Substances (TBARS)

Myocardial tissue was homogenized (10% wt/vol) in 1.15% KCL solution (pH 7.4). The homogenate (0.1 ml) was mixed with 50 µl, 0.8% butylated hydroxytoluene solution in glacial acetic acid, 0.20 ml of 8.1% sodium dodecyl sulfate (SDS) solution, 1.50 ml of 20% acetic acid solution adjusted at pH 3.5 with 10 mol/L NaOH, 0.70 ml water and 1.50 ml of 0.8% thiobarbituric acid solution. The mixture was kept at 5°C for 60 min and then heated at 100°C for 60 min. After cooling, the mixture was extracted with 1.0 ml water and 5.0 ml *n*-butanol:pyridine (15:1 v/v) and centrifuged at 700 G for 10 min. The absorbance of the organic phase at 532 nm was measured (220 A spectrophotometer, HITACHI, Tokyo, Japan). The amount of TBARS was determined by the absorbance with the molecular extinction coefficient of 156,000 and expressed as nmol/g wet tissue weight.^{17,18}

Table 1 Effect of Allopurinol on Left Ventricular Function Before and After Ischemia-Reperfusion

	Before ischemia		30 min after reperfusion	
	Control (n=5)	Allopurinol (n=5)	Control (n=5)	Allopurinol (n=5)
LVPmax (mmHg)	107.0±1.7	106.2±3.2	96.1±2.9*	110.5±5.3†
LVEDP (mmHg)	5.0±0.1	5.1±0.1	39.1±6.0*	23.1±3.2*†
Developed pressure (mmHg)	102.0±1.7	101.1±3.3	57.0±5.8*	87.4±5.7*†
dP/dtmax (mmHg/s)	2,982±63	2,936±78	1,284±156*	1,949±130*†
dP/dtmin (mmHg/s)	-1,903±59	-1,766±69	-848±78*	-1,353±99*†

LVPmax, peak left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; dP/dtmax, peak rate of rise of LVP; dP/dtmin, peak rate of fall of LVP.

* $p < 0.05$ vs before ischemia, † $p < 0.05$ vs control.

Measurement of ROS in Myocardial Tissue

ROS were quantified in tissues by ESR spectroscopy with hydroxy-TEMPO as previously described.¹⁹ In brief, myocardial tissue was homogenized in 50 mmol/L sodium phosphate buffer (pH 7.4) containing protease inhibitors. First, the homogenate was immediately reacted with hydroxy-TEMPO (0.1 mmol), and its ESR spectra were recorded using an X-band (9.45-GHz) ESR spectrometer (JES-FA100/BU, JEOL, Tokyo, Japan). Second, dimethylthiourea (DMTU; 50 mmol/L), a specific OH⁻ scavenger, was also added to the reaction mixture and we measured the decrease in signal decay after the addition of DMTU, which indicates the amount of hydroxyl radicals.

Statistical Analysis

Comparisons among multiple groups were determined by two-way analysis of variance with Bonferroni's post hoc test. Comparisons for values between the control and allopurinol groups were performed by unpaired Student's t-test as appropriate. All data are expressed as the mean value ± SEM; $p < 0.05$ was considered statistically significant.

Results

Effect of Allopurinol on LVP

LVP, peak LVP (LVPmax), left ventricular end-diastolic pressure (LVEDP), developed pressure, peak rate of rise of LVP (dP/dtmax) and peak rate of fall of LVP (dP/dtmin) before global ischemia and after reperfusion in the control and allopurinol groups are summarized in Table 1. There were no significant differences in LVPmax, LVEDP, developed pressure, dP/dtmax or dP/dtmin before ischemia between the control and allopurinol groups, suggesting that allopurinol had no significant effect on left ventricular function before ischemia. After ischemia, LVPmax, developed pressure, dP/dtmax and dP/dtmin were significantly decreased and LVEDP was significantly increased in both the control and allopurinol group; however, in hearts treated with allopurinol, the changes in LVPmax, developed pressure, dP/dtmax and dP/dtmin and LVEDP were significantly attenuated in comparison with those of control hearts ($p < 0.05$). These data suggest that allopurinol significantly improved left ventricular function after ischemia-reperfusion.

Effect of Allopurinol on LDH Activity

We measured LDH activity in the coronary effluent as an index of myocardial cellular injury after ischemia-reperfusion. Fig 1 shows the time course of LDH in the coronary effluent before and after ischemia-reperfusion. LDH activity significantly increased after reperfusion and

Fig 1. Effect of allopurinol on lactate dehydrogenase activity before ischemia and after reperfusion. * $p < 0.05$ vs control.

peaked between 5 and 10 min after reperfusion in both groups. In the allopurinol group, LDH activity was significantly lower at 5 min, 10 min and 15 min after reperfusion compared with the control group (5 min: 0.39 ± 0.14 and 0.98 ± 0.22 , 10 min: 0.30 ± 0.16 and 1.04 ± 0.19 , 15 min: 0.10 ± 0.04 and 0.42 ± 0.09 IU·min⁻¹·g⁻¹, respectively, $p < 0.05$). Therefore, allopurinol significantly decreased the release of LDH from the myocardium, suggesting attenuation of the myocardial damage caused by ischemia.

Effect of Allopurinol on Adenine Nucleotides, and Purine Metabolites in Myocardium

Fig 2 shows the time course of the ATP, ADP, AMP, IMP, inosine, hypoxanthine and xanthine concentrations in myocardial tissue before and after ischemia-reperfusion. There were no significant differences in these concentrations before global ischemia between the control and allopurinol groups. The concentrations of ATP, ADP, AMP and IMP significantly decreased after reperfusion in both the control and allopurinol groups, with no differences between the 2 groups (Fig 2A–D). The concentrations of inosine, hypoxanthine and xanthine significantly increased after reperfusion and gradually decreased during reperfusion (Fig 2E–G). The concentration of xanthine in the allopurinol group was significantly lower after reperfusion compared with the control (0 min: 0.191 ± 0.041 and 0.076 ± 0.014 , 1 min: 0.103 ± 0.012 and 0.051 ± 0.010 , 5 min: 0.075 ± 0.017 and 0.025 ± 0.007 μmol/g, respectively, $p < 0.05$) whereas there were no significant differences in the concentration of inosine or hypoxanthine between the 2 groups. Taken together, these results suggest that allopurinol does not preserve myocardial ATP; however, it reduces the myocardial concentration of xanthine.

Fig 2. Time course of the concentrations of ATP, ADP, AMP, IMP, inosine, hypoxanthine and xanthine during ischemia and reperfusion. * $p < 0.05$ vs control, † $p < 0.05$ vs pre-ischemia; $n = 5-7$ at each point.

Fig 3. Effect of allopurinol on xanthine oxidase (XO) activity during ischemia and reperfusion. * $p < 0.05$ vs control.

Effect of Allopurinol on Xanthine Oxidase Activity During Ischemia-Reperfusion

In order to determine whether or not xanthine oxidase activity is increased after ischemia and reperfusion and is suppressed by allopurinol, we measured the enzyme activity in the myocardium before and after ischemia-

Fig 4. Effect of allopurinol on the production of TBARS in myocardial tissue 30 min after ischemia-reperfusion. * $p < 0.05$ vs control.

reperfusion. Fig 3 shows the time course of xanthine oxidase activity before and after ischemia-reperfusion. Xanthine oxidase activity did not change significantly after ischemia-reperfusion; however, the enzyme activity in hearts treated with allopurinol was significantly decreased compared with the control hearts before and after ischemia-reperfusion. Thus, the xanthine oxidase activity did not change after ischemia-reperfusion, but it was significantly inhibited by allopurinol.

Effect of Allopurinol on the Production of TBARS After Ischemia–Reperfusion

Lipid peroxidation products were measured as a marker of accumulation of oxidative injury. Fig 4 shows the production of TBARS after reperfusion in control and allopurinol-treated hearts. TBARS production was significantly lower in hearts treated with allopurinol than in control hearts (5.6 ± 1.5 and 13.0 ± 2.3 nmol/g, respectively, $p < 0.05$).

Effect of Allopurinol on Hydroxyl Radicals Generation After Ischemia–Reperfusion

Fig 5 shows the time course of ESR signal decay of hydroxy-TEMPO just before and after reperfusion in the control and allopurinol groups. There were no significant differences in ESR signal decay of hydroxy-TEMPO just before reperfusion between the control and allopurinol groups. In the control group, the ESR signal decay of hydroxy-TEMPO was significantly increased at 1 min after reperfusion and then gradually decreased throughout reperfusion. On the other hand, allopurinol significantly suppressed the increased signal decay at 1 min and 5 min compared with the control group (1 min: 0.028 ± 0.003 and 0.019 ± 0.002 , 5 min: 0.018 ± 0.002 and 0.007 ± 0.003 , respectively, $p < 0.05$). These data suggest that allopurinol significantly reduces the generation of hydroxyl radicals after ischemia–reperfusion.

Discussion

The results of the present study show that myocardial hydroxyl radicals significantly increased after ischemia–reperfusion and significantly decreased in allopurinol-treated animals; moreover this decrease was accompanied by improved cardiac function after ischemia–reperfusion. In addition, the production of TBARS was also suppressed by allopurinol. Furthermore, allopurinol significantly inhibited myocardial xanthine oxidase activity and decreased the myocardial concentration of xanthine after ischemia–reperfusion.

The present findings suggest that allopurinol prevents the myocardial dysfunction caused by ischemia–reperfusion injury by suppressing ROS. It has been proposed that xanthine oxidase plays an important role in the generation of free radicals after ischemia–reperfusion^{3,4,11,20,21} and that xanthine oxidase inhibitors have protective effects against ischemia–reperfusion injury^{3–10}. However, it is still unclear whether the reduction of free radicals is involved in the beneficial effect of allopurinol after ischemia–reperfusion. Using ESR spectroscopy with 5,5-dimethyl-1-pyrroline-N-oxide, Thompson-Gorman et al demonstrated that oxypurinol significantly decreased ROS in the coronary effluent after ischemia–reperfusion²². On the other hand, Pisarenko et al failed to demonstrate the decrease of ROS in the coronary effluent with allopurinol using the same method²³. In those studies, myocardial free radicals were not directly measured. Thus, ours is the first report to demonstrate myocardial ROS generation after ischemia–reperfusion using ESR with hydroxy-TEMPO.

ATP, ADP, AMP and IMP significantly decreased, whereas inosine, hypoxanthine and xanthine significantly increased after ischemia, which means that ATP degradation occurs in the hearts during ischemia followed by accumulation of the purine metabolites that are sources of ROS generation (ie, inosine, hypoxanthine and xanthine).

Fig 5. Effect of allopurinol on hydroxyl radical generation after ischemia–reperfusion. * $p < 0.05$ vs control, † $p < 0.05$ vs 0 min.

However, our results showed that allopurinol did not retain ATP and did not affect the concentrations of these purine metabolites, except for that of xanthine, after ischemia–reperfusion, which suggests that the beneficial effects of allopurinol on myocardial dysfunction after ischemia–reperfusion are not mediated by the salvage of adenine nucleotides, consistent with the results of a previous study²². On the other hand, it has been reported that ATP is decreased and AMP increased during ischemia^{24,25} and that xanthine oxidase inhibitors can improve post-ischemic left ventricular function via the salvage and preservation of adenine nucleotides^{23,26,27}. These discrepant results might be attributed to different experimental protocols. In our study long-term ischemia may have led to a rapid degradation of purine metabolites followed by a decrease in AMP, whereas the perfusion of allopurinol throughout the entire experiment or the combination with long-term pretreatment prior to heart excision may have increased the efficiency of ATP salvage in the previous study²³ compared with the acute administration of the drug prior to and after ischemia in the present study. The shorter duration of ischemia (10–15 min) in previous studies may also account for the increased efficiency of ATP salvage^{26,27}. Further studies are needed to clarify the involvement of energy preservation in the protective effect of allopurinol on myocardial dysfunction after ischemia–reperfusion.

The increase in the activity of xanthine oxidase after ischemia–reperfusion has been proposed as a key role in the generation of free radicals^{1,28}. It has been suggested that xanthine dehydrogenase is converted to xanthine oxidase during ischemia²⁹ and xanthine oxidase activity has been found to increase relative to dehydrogenase during ischemia²². However, the activity of xanthine oxidase did not significantly change throughout the entire experiment in the present study, whereas it was completely inhibited by allopurinol. In addition, we confirmed using Western blot that the amount of myocardial xanthine oxidase was not significantly affected by allopurinol before or after ischemia–reperfusion (data not shown). No significant conversion of xanthine dehydrogenase to xanthine oxidase has been previously observed in ischemic rat hearts^{30,31} and the alteration in xanthine oxidase activity is not the major limiting factor in the process of free radicals generation during postischemic reperfusion²¹. In the present study, myocardial substrate (ie, inosine, hypoxanthine and xanthine) levels rapidly decreased after reperfusion, proba-

bly because of both substrate metabolism and washout, followed by decreases in hydroxyl radicals (Fig 5). These results indicate that an increase in the substrate of xanthine oxidase, rather than an increased xanthine oxidase activity or the amount of xanthine oxidase, is responsible for the generation of free radicals after ischemia–reperfusion. Further studies will be needed to clarify which mechanism is more important for the decrease in ROS associated with allopurinol treatment.

Although the level of xanthine oxidase activity is significant in the myocardium of rats and dogs, it is relatively low in the myocardium of rabbits and humans^{32,33} which makes it unclear whether the same principle of xanthine oxidase inhibition can be applied to human myocardial protection against ischemia–reperfusion injury. Some reports deny the protective effect of allopurinol on ischemia–reperfusion injury in rabbit hearts;^{34,35} however, Terada et al reported that allopurinol improved left ventricular function in rabbit hearts.⁷ In addition, pretreatment with allopurinol has been shown to improve postoperative recovery^{9,10} and reduce lipid peroxidation⁹ in patients undergoing open-heart surgery; those reports suggest that allopurinol may contribute to myocardial protection after ischemia–reperfusion in humans. Immunohistochemistry of human tissue has revealed that xanthine oxidase is mainly localized in the vascular endothelium and smooth muscle cells.^{36,37} Thus, xanthine oxidase from coronary endothelial/smooth muscle cells may contribute to the production of free radicals after ischemia–reperfusion. On the other hand, Saugstad postulated that xanthine oxidase originates from other organs, such as the liver and intestines, reaches the heart and produces free radicals under hypoxic conditions.³³ In addition, it has been reported that circulating plasma xanthine oxidase contributes to vascular endothelium dysfunction.³⁸ Taking those findings together, it is possible that xanthine oxidase originating from other organs contributes to the generation of free radicals in the heart after ischemia reperfusion; however, it is unclear to what extent circulating xanthine oxidase is important for ischemia–reperfusion injury. Further studies are necessary to clarify the exact mechanism of myocardial ischemia–reperfusion injury and the protection of the myocardium by allopurinol in various species.

In conclusion, allopurinol attenuated myocardial dysfunction in the rat heart after ischemia–reperfusion by suppressing ROS generation, not by the salvage of ATP. This is the first report to directly measure myocardial ROS and thus demonstrate the clinical importance of reducing them ischemia–reperfusion injury. Allopurinol has a well-established safety profile and is used widely for the treatment of gout. It has, therefore, the potential to be developed as a novel therapeutic strategy for ischemia–reperfusion injury.

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