



Q50, an Iron-Chelating and Zinc-Complexing Agent, Improves Cardiac Function in Rat Models of Ischemia/Reperfusion-Induced Myocardial Injury

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Background: Reperfusion of ischemic myocardium may contribute to substantial cardiac tissue damage, but the addition of iron chelators, zinc or zinc complexes has been shown to prevent heart from reperfusion injury. We investigated the possible beneficial effects of an iron-chelating and zinc-complexing agent, Q50, in rat models of ischemia/reperfusion (I/R)-induced myocardial infarction and on global reversible myocardial I/R injury after heart transplantation.

Methods and Results: Rats underwent 45-min myocardial ischemia by left anterior descending coronary artery ligation followed by 24 h reperfusion. Vehicle or Q50 (10 mg/kg, IV) were given 5 min before reperfusion. In a heart transplantation model, donor rats received vehicle or Q50 (30 mg/kg, IV) 1 h before the onset of ischemia. In myocardial infarcted rats, increased left ventricular end-systolic and end-diastolic volumes were significantly decreased by Q50 post treatment as compared with the sham group. Moreover, in I/R rat hearts, the decreased dP/dt_{max} and load-independent contractility parameters were significantly increased after Q50. However, Q50 treatment did not reduce infarct size or have any effect on increased plasma cardiac troponin-T-levels. In the rat model of heart transplantation, 1 h after reperfusion, decreased left ventricular systolic pressure, dP/dt_{max} , dP/dt_{min} and myocardial ATP content were significantly increased and myocardial protein expression of superoxide dismutase-1 was upregulated after Q50 treatment.

Conclusions: In 2 experimental models of I/R, administration of Q50 improved myocardial function. Its mechanisms of action implicate in part the restoration of myocardial high-energy phosphates and upregulation of antioxidant enzymes. (*Circ J* 2013; **77**: 1817–1826)

Key Words: Antioxidants; Ischemia/reperfusion; Myocardial infarction; Transplantation

Myocardial ischemia/reperfusion (I/R) injury is associated with a variety of cardiovascular diseases, but particularly after invasive procedures, such as percutaneous coronary angioplasty, stents application, coro-

nary bypass surgery, heart transplantation or following acute myocardial infarction (MI). Restoration of blood flow to the ischemic myocardium is necessary to salvage cardiomyocytes from eventual death, but it can itself induce injury, thereby

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reducing the beneficial effects of myocardial reperfusion.¹ Four types of reperfusion injury have been described in the literature. In the clinical setting, reperfusion injury is manifested by myocardial stunning,² which occurs after reperfusion of a globally ischemic myocardium, or in the setting of regional I/R, reperfusion arrhythmias,³ including ventricular arrhythmias such as ventricular tachycardia and fibrillation, myocyte death and necrosis,⁴ and endothelial- and microvascular dysfunction (including the no-reflow phenomenon).⁵

Left ventricular (LV) remodeling starts immediately after acute MI and may promote progressive enlargement of the LV to develop the chronic phase of heart failure (HF).⁶ One possible treatment of end-stage HF is heart transplantation. I/R injury is a common condition during cardiac surgery, which is recognized as a major determinant of primary graft dysfunction.⁷ Therefore, the prevention of cardiomyocyte loss from I/R insult has particularly important implications in the interventional treatment of coronary ischemia and also in cardiac surgery for cardiopulmonary bypass and heart transplantation. During reperfusion, the acutely ischemic myocardium is subjected to several abrupt biochemical and metabolic changes, including intracellular calcium overload, energy depletion, acidosis and generation of reactive oxygen species (ROS).^{8,9} All of these changes interact to mediate apoptosis, autophagy and necrosis.¹⁰⁻¹² One of the main sources of ROS generation is neutrophil granulocytes, which cause myocardial inflammation, but endothelial cells and cardiomyocytes can also generate ROS at the time of reperfusion.¹³ Oxidative stress during myocardial reperfusion reduces the bioavailability of the intracellular signaling molecule, nitric oxide thereby removing its cardioprotective effects.¹² It has been proposed that chelation of ferric iron protects against myocardial I/R injury^{14,15} and in addition, several lines of evidence indicate that antioxidant drugs or upregulation of endogenous antioxidant defense mechanisms could protect the tissues against reperfusion injury.⁶ The role of zinc in the antioxidant pathway shows promise as a target for new cardioprotective therapies. Powell et al showed that zinc ions protect isolated rat hearts from I/R injury through inhibition of oxidative stress.¹⁶ Additionally, iron in its redox and active form represents the main mediator for the formation of the free radicals that contribute to oxidative stress. Its chelation makes it unavailable for this type of generation. Therefore, the use of an agent possessing both iron-chelating and zinc-complexing properties may be a new concept in cardioprotection against I/R injury. Substances that belong to the 8-hydroxyquinoline family have been shown to have zinc-complexing properties,¹⁷ and 8-hydroxyquinolines are also documented as iron chelators.¹⁸ Q50 belongs to the family of 8-hydroxyquinolines and may be a good candidate because it chelates iron and additionally acts on the intracellular source of zinc forming a protective complex.

Therefore, in the present study, we investigated the potential beneficial effects of Q50, an iron-chelating and zinc-complexing agent, in rat models of MI induced by regional I/R as well as global myocardial I/R injury after heart transplantation.

Methods

Animals

Male Lewis and Sprague-Dawley rats (250–350 g; Charles River, Sulzfeld, Germany) were housed in a room at 22±2°C under 12-h light/dark cycles and were fed a standard laboratory rat diet and water ad libitum. The rats were acclimatized for at least 1 week before experiments. All animals received

humane care in compliance with the ‘Principles of Laboratory Animal Care’ formulated by the National Society for Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the Institute of Laboratory Animal Resources and published by the US National Institutes of Health (NIH Publication No. 86-23, revised 1996). This investigation was reviewed and approved by the appropriate institutional review committees.

Rat Model of Myocardial I/R Injury

Surgical Preparation of Regional I/R Rats were anesthetized with sodium pentobarbital (60 mg/kg IP). An intratracheal tube was inserted, and the animals were artificially ventilated using a rodent ventilator (Föhr Medical Instruments, Seeheim-Ober Beerbach, Germany). The body temperature was maintained at 37°C with a controlled heating pad. The chest was opened via a left thoracotomy, followed by a pericardiotomy. A 6-0 single silk suture was passed around the left anterior descending coronary artery (LAD) and the ends of the tie were pulled through a small pledget to form a snare and then tightened. After 45 min of ischemia, reperfusion was achieved by releasing the snare. After surgery, the thorax was closed, the skin was sutured and the rats were allowed to recover on a heating pad. Sham-operated animals were subjected to the same surgical procedures, except that the suture around the LAD coronary artery was not tied.

Experimental Groups Sprague-Dawley rats were randomized into 4 groups of 6–8 rats: (1) Sham: animals received vehicle but no tightening of the coronary suture, (2) Sham+Q50: rats received Q50 and the ligature placed around the LAD but without occlusion, (3) I/R: rats were treated with vehicle and subjected to I/R, and (4) I/R+Q50: animals were given Q50 and subjected to I/R. Rats underwent 45 min of myocardial ischemia followed by 24 h of reperfusion. Vehicle (10% Solutol® HS15) or Q50 (10 mg/kg) were given as an intravenous bolus 5 min before the onset of reperfusion. The dose of Q50 was chosen on the basis of our pilot studies.

In Vivo Hemodynamic Parameters After 24 h of reperfusion, the rats were anesthetized with sodium pentobarbital (60 mg/kg IP), tracheotomized, intubated and artificially ventilated. LV pressure-volume analysis to assess cardiac function was performed with a 2F microtip pressure-volume catheter (SPR-838, Millar Instruments, Houston, TX, USA) as described previously¹⁹ ([Supplementary File 1](#)).

Determination of Area at Risk and Infarct Size After hemodynamic measurements, the hearts were excised and quickly attached to a Langendorff apparatus. Next, 1.5 ml of Evans blue dye (1% w/v) was injected into the aorta and coronary arteries to demarcate the ischemic risk (non-stained) and non-risk (stained) areas of the heart. Heart tissue was excised and transverse slices were incubated with 1% TTC (2,3,5-triphenyltetrazolium chloride) for 30 min at 37°C ([Supplementary File 1](#)).

Biochemical Estimation Blood collected from the rats into EDTA tubes was immediately centrifuged, and the plasma separated. Cardiac troponin-T concentrations were determined by automatic biochemistry analyzer.

Rat Model of Heterotopic Heart Transplantation

Surgical Preparation of Heart Transplantation Transplantations were performed in an isogeneic Lewis to Lewis rat strain, so organ rejection was not expected. The experimental model was established according the reported method.⁷ Briefly, the donor rats were anaesthetized intraperitoneally with a mixture of ketamine (100 mg/kg) and xylazine (3 mg/kg) and

heparinized (400 IU/kg). Cardiac arrest was induced by Custodiol (histidine-tryptophan-ketoglutarate) solution. After 1 h of ischemia, the hearts were implanted intra-abdominally, anastomosing the aorta and pulmonary artery of the donor heart with the abdominal aorta and inferior caval vein of the recipient, respectively (**Supplementary File 1**).

Experimental Groups Rats were randomly divided into 4 groups: (1) control: heart explanted without any treatment, (2) control + Q50: Q50 administered 1 h prior to explantation, (3) I/R: donor rats received vehicle 1 h prior to explantation, then hearts subjected to 1 h ischemia and transplanted, and (4) Q50 + I/R: Q50 treatment of the donor animals 1 h prior to explantation, then hearts subjected to 1 h ischemia and transplanted. Vehicle (10% Solutol® HS15) or Q50 (30 mg/kg) were given intravenously. There were 6 male Lewis donor and 6 recipient rats in each group and for each measurement.

Hemodynamic Measurements After 1 h of reperfusion, rats were anaesthetized intraperitoneally with a mixture of ketamine (100 mg/kg) and xylazine (3 mg/kg), and a 3F latex balloon catheter (Edwards Lifesciences Corporation, Irvine, CA, USA) was introduced into the LV via the apex to determine LV systolic pressure, LV end-diastolic pressure, maximal slope of the systolic pressure increment, dP/dt_{max} , and the maximal slope of the diastolic pressure decrement, dP/dt_{min} , by a Millar micromanometer (Millar Instruments, Houston, TX, USA) at different LV volumes. From these data, LV pressure-volume relationships were constructed using PVAN 3.6 software (Millar Instruments, Houston, TX, USA).

Determination of High-Energy Phosphate Levels For this analysis, 1 g of heart tissue was homogenized and centrifuged. Next, 5 ml of supernatant was neutralized with 1 ml of triethanolamine-HCl/K₂CO₃ solution. ATP degradation was assessed with standard photometry. Using an enzyme kinetic assay, the content of each of ATP, ADP and AMP was expressed as micromoles per gram of dry weight. The energy charge potential was calculated as: $(ATP + 0.5ADP)/(ATP + ADP + AMP)$ (**Supplementary File 1**).

Quantitative Real-Time Polymerase Chain Reaction Total RNA was isolated from each heart with the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany). RNA concentration and purity was determined photometrically (260, 280 and 230 nm) (**Supplementary File 1**).

Western Blotting Myocardial proteins were extracted into a solution containing 8 mol/L urea, 5 mmol/L EDTA, 0.002% trasyolol, 0.05 mmol/L PMSF, 0.003% triton X-100 containing protease inhibitors (Roche, Mannheim, Germany). Protein concentration was determined by a commercial kit according to the manufacturer's protocol (BCA Protein Assay Kit; Thermo Scientific, Rockford, IL, USA) (**Supplementary File 1**).

Cardiac Myocyte Protection Studies In Vitro

H9c2 rat embryonic cardiac muscle cells (ATCC, Rockville, MD, USA) were cultured in Dulbecco's Modified Eagle's Medium and treated with and without Q50 (5 μ mol/L) 30 min after exposure of hydrogen peroxide (H₂O₂; 100 μ mol/L). Total RNA was extracted using the RNeasy Mini Kit (Qiagen) and cDNA synthesis was performed with the cDNA Archive kit (Applied Biosystems, Foster City, CA, USA) (**Supplementary File 1**).

A real-time cell electronic sensing cardioprotection assay was performed as described previously with slight modifications²⁰ (**Supplementary File 1**). On the following day, H9c2 rat embryonic cardiac muscle cells were post-treated (30 min after H₂O₂ treatment) with Q50 or solvent (dimethyl sulfoxide; negative control cells) of the compound. The absolute control

group did not receive H₂O₂ treatment. The H₂O₂ concentration used here (100 μ mol/L) to elicit cell injury was previously optimized for H9c2 cells according to their sensitivity to oxidative stress. Cells were dynamically monitored over 24 h by measuring the electrical impedance every 5 min. The raw plate reads for each titration point were normalized relative to the cell index status immediately before the addition of H₂O₂.

Measurement of Activities of Human Matrix Metalloproteinase (MMP) Enzymes

The SensoLyte® MMP Assay Kit was used for the continuous spectrophotometric assay of MMP-2 and MMP-9 activities according to the manufacturer's protocol (Anaspec Inc, San Jose, CA, USA).

Chemical Reagents

Q50 was synthesized at Avidin Ltd (Szeged, Hungary), dissolved in 10% Solutol® HS15, a polyethylene glycol 660 hydroxystearate as a nonionic solubilizer for injection solutions. Custodiol was purchased from Dr Franz Köhler Chemie GmbH (Alsbach-Hähnlein, Germany).

Statistical Analysis

All data are expressed as mean \pm SEM. In the case of heart transplantation hemodynamic parameters, Student's t-test was used to analyze the differences between groups. In all other case, means between groups were compared by 1-way ANOVA with Bonferroni correction for multiple comparisons. $P < 0.05$ was considered statistically significant.

Results

Effect of Q50 Post-Treatment on Regional Myocardial I/R Injury (Post-MI)

Effect of Q50 on Infarct Size In rats subjected to coronary artery occlusion and reperfusion, there was no difference in the area at risk between the vehicle- and Q50-treated rats, indicating that a comparable degree of ischemia was induced in both groups. Posts ischemic treatment with Q50 did not reduce infarct size compared with the I/R group (I/R + Q50: $43 \pm 12\%$ vs. I/R: $41 \pm 6\%$, $P > 0.05$).

Effect of Q50 on Plasma Cardiac Troponin-T Levels After 24 h of reperfusion, the levels of plasma cardiac troponin-T in the I/R group were significantly increased compared with the sham and sham + Q50 groups (I/R: $2,820 \pm 584$ pg/ml vs. sham: 487 ± 118 pg/ml vs. sham + Q50: 399 ± 114 pg/ml, $P < 0.05$). Posts ischemic treatment with Q50 did not significantly decrease plasma levels of this enzyme (I/R + Q50: $2,210 \pm 784$ pg/ml).

Effect of Q50 on Cardiac Function Cardiac parameters derived from the pressure-volume analysis comparing MI rats with controls are shown in **Table 1**. There were no significant differences in heart rate, LV end-diastolic pressure, stroke volume, cardiac output, stroke work (SW) or the slope of the end-diastolic pressure-volume relationship (EDPVR) values between the groups (**Table 1**). However, the increased end-systolic and end-diastolic volumes in the MI rats were significantly reduced after posts ischemic treatment with Q50. In the I/R group, decreased LV load-dependent (dP/dt_{max}) and decreased load-independent (slope of dP/dt_{max} /end-diastolic volume relationship and maximum time-varying elastance) contractility parameters were significantly increased after posts ischemic treatment with Q50 (**Table 1, Figures 1A–C**). Moreover, the ejection fraction was significantly increased in the I/R + Q50 group when compared with the I/R group (**Table 1**).

Systolic and diastolic blood pressures and mean arterial

Parameter	Sham	Sham + Q50	I/R	I/R + Q50
Basic hemodynamic data				
Heart rate (beats/min)	404±8	431±19	383±17	410±21
Systolic blood pressure (mmHg)	157±3	139±4* [#]	116±3*	129±7*
Diastolic blood pressure (mmHg)	126±3	109±3* [#]	93±4*	102±5*
Mean arterial pressure (mmHg)	136±3	119±3* [#]	101±3*	111±6*
LV pressure and volume				
LV end-systolic pressure (mmHg)	136±8	127±5 [#]	110±3*	118±8
LV end-diastolic pressure (mmHg)	15±2	14±3	16±2	15±4
End-systolic volume (μl)	52±10	64±20	93±8*	43±8 [#]
End-diastolic volume (μl)	113±17	120±19 [#]	177±15*	97±13 [#]
Stroke volume (μl)	61±24	85±7	84±10	86±9
Ejection phase and pressure-volume relationship indexes				
Ejection fraction (%)	46±15	51±8	47±3	52±10 [#]
Cardiac output (ml/min)	46±6	38±4	50±9	35±3
Stroke work (mmHg/μl)	10,129±1,895	8,373±1,838	11,790±2,031	6,762±1,623
PRSW (mmHg)	93±14	114±12 [#]	75±4*	93±11
Indexes of the active phase of relaxation				
dP/dt _{min} (mmHg/s)	12,625±1678	11,910±1,119 [#]	7,217±275*	8,653±967
Tau (ms)	10.4±0.9	10.4±1.1 [#]	14.6±0.7*	13.9±1.1*
Index of the passive phase of relaxation				
Slope of EDPVR (mmHg/μl)	0.043±0.011	0.070±0.007	0.062±0.011	0.050±0.008

*P<0.05 vs. sham, #P<0.05 vs. I/R.

I/R, ischemia/reperfusion; LV, left ventricular; PRSW, preload recruitable stroke work; dP/dt_{min}, maximal slope of the diastolic pressure decrement; Tau, time constant of LV pressure decay; EDPVR, end-diastolic pressure-volume relationship.

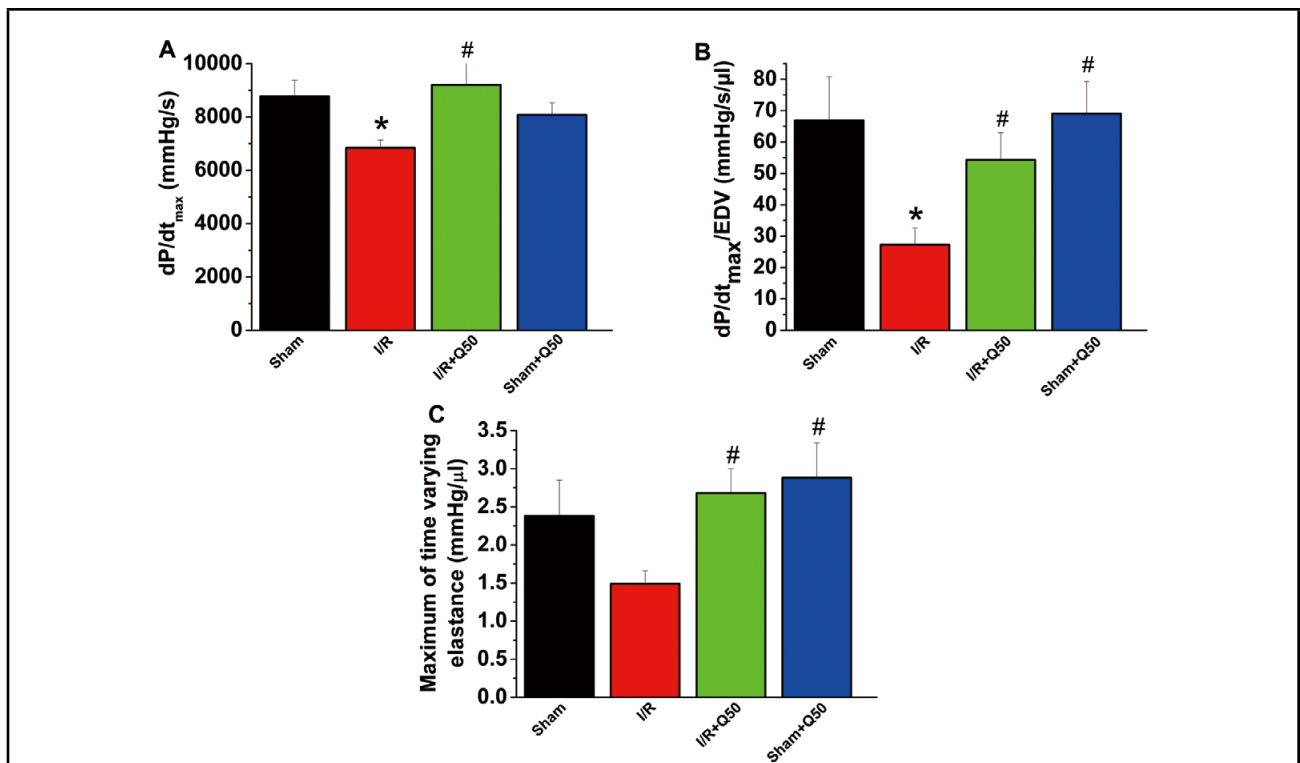


Figure 1. Effect of Q50 on left ventricular contractility in a rat model of myocardial infarction. (A) Maximal slope of the systolic pressure increment dP/dt_{max}, (B) dP/dt_{max}/end-diastolic volume (EDV) and (C) time-varying elastance in rats subjected to a 45-min occlusion of the left anterior descending coronary artery followed by a 24 h reperfusion. I/R, ischemia/reperfusion. *P<0.05 vs. sham; #P<0.05 vs. I/R.

pressure were significantly reduced in the I/R, I/R+Q50 and sham+Q50 groups as compared with sham-operated rats (Table 1). When compared with the sham group, rats with MI showed significantly decreased LV end-systolic pressure, pre-load recruitable SW, dP/dt_{min} and impaired cardiac relaxation, as reflected by prolonged Tau. Postischemic treatment with Q50 did not significantly restore these parameters (Table 1).

Effect of Q50 Pretreatment on Global I/R Injury (Post-Heart Transplantation)

Effect of Q50 on Graft Function After 1 h of reperfusion, LV systolic pressure and dP/dt_{max} were significantly increased in the Q50-treated group compared with the I/R group, indicating improved myocardial contractility (Table 2, Figures 2A,B). Moreover, Q50 treatment resulted in a significant increase in dP/dt_{min} values compared with the I/R group, reflecting improved myocardial relaxation (Table 2). LVEDP, as a marker of the standardized balloon-catheter measurements, did not show any major differences (Table 2, Figure 2C).

Effect of Q50 on Graft Myocardial High-Energy Phosphate Content Myocardial high-energy phosphate content and the ATP and ADP levels were preserved by Q50 preconditioning as compared with the I/R group (Table 3). AMP level did not show any relevant changes between the groups. The energy

Table 2. Effect of Q50 on Graft Function After Heart Transplantation

Parameter	I/R	Q50+I/R
LVSP (mmHg)	80±2	105±5*
dP/dt_{max} (mmHg/s)	1,781±94	3,219±190*
LVEDP (mmHg)	5.5±2.0	7.1±4.1
dP/dt_{min} (mmHg/s)	989±115	2,477±424*

* $P < 0.05$ vs. I/R.

LVSP, dP/dt_{max} , LVEDP and dP/dt_{min} at an intraventricular volume of $80 \mu\text{l}$ 1 h after reperfusion.

dP/dt_{max} , maximal slope of the systolic pressure increment; LVEDP, LV end-diastolic pressure; LVSP, LV peak systolic pressure. Other abbreviations as in Table 1.

charge potential, as an indicator of the myocardial energy level, showed a significant improvement in the Q50-pretreated rats when compared with the I/R group.

Effect of Q50 on Graft Gene Expression Quantitative real-time polymerase chain reaction of myocardial RNA extracts revealed that relative mRNA expression for superoxide dismutase (SOD)-1 and cytochrome-c oxidase remained unchanged in the control, control-Q50 and I/R groups. However, their expressions were significantly upregulated after Q50 treat-

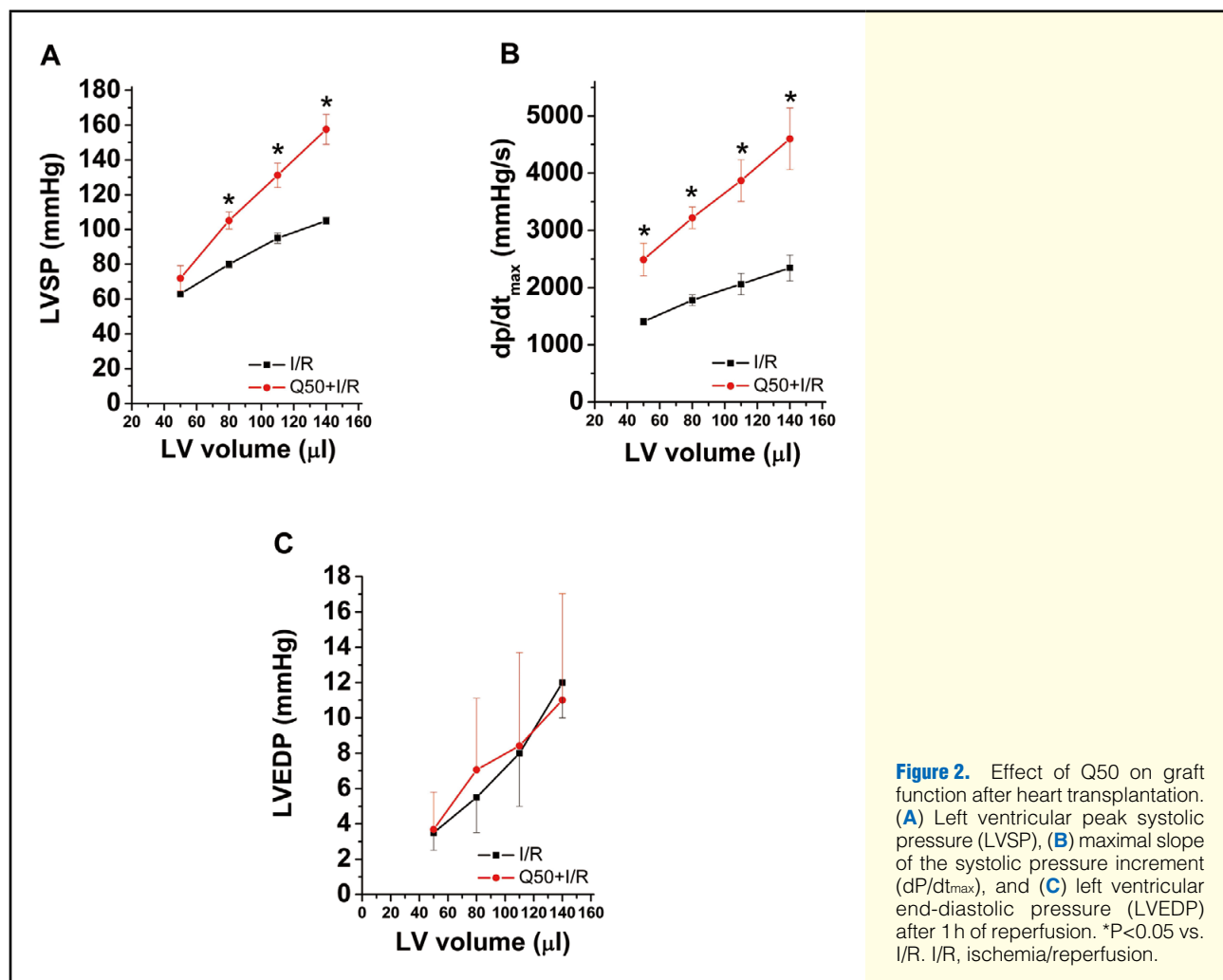


Figure 2. Effect of Q50 on graft function after heart transplantation. (A) Left ventricular peak systolic pressure (LVSP), (B) maximal slope of the systolic pressure increment (dP/dt_{max}), and (C) left ventricular end-diastolic pressure (LVEDP) after 1 h of reperfusion. * $P < 0.05$ vs. I/R. I/R, ischemia/reperfusion.

Parameter	Control	I/R	Q50+I/R
ATP ($\mu\text{mol/g}$)	6.58 \pm 1.12	1.86 \pm 0.41*	6.66 \pm 0.63
ADP ($\mu\text{mol/g}$)	3.48 \pm 0.16	2.05 \pm 0.42*	5.01 \pm 0.43
AMP ($\mu\text{mol/g}$)	1.91 \pm 0.22	2.07 \pm 0.22	2.59 \pm 0.61
Energy charge potential	0.69 \pm 0.07	0.49 \pm 0.04*	0.85 \pm 0.08

*P<0.05 vs. other groups.

I/R, ischemia/reperfusion; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate.

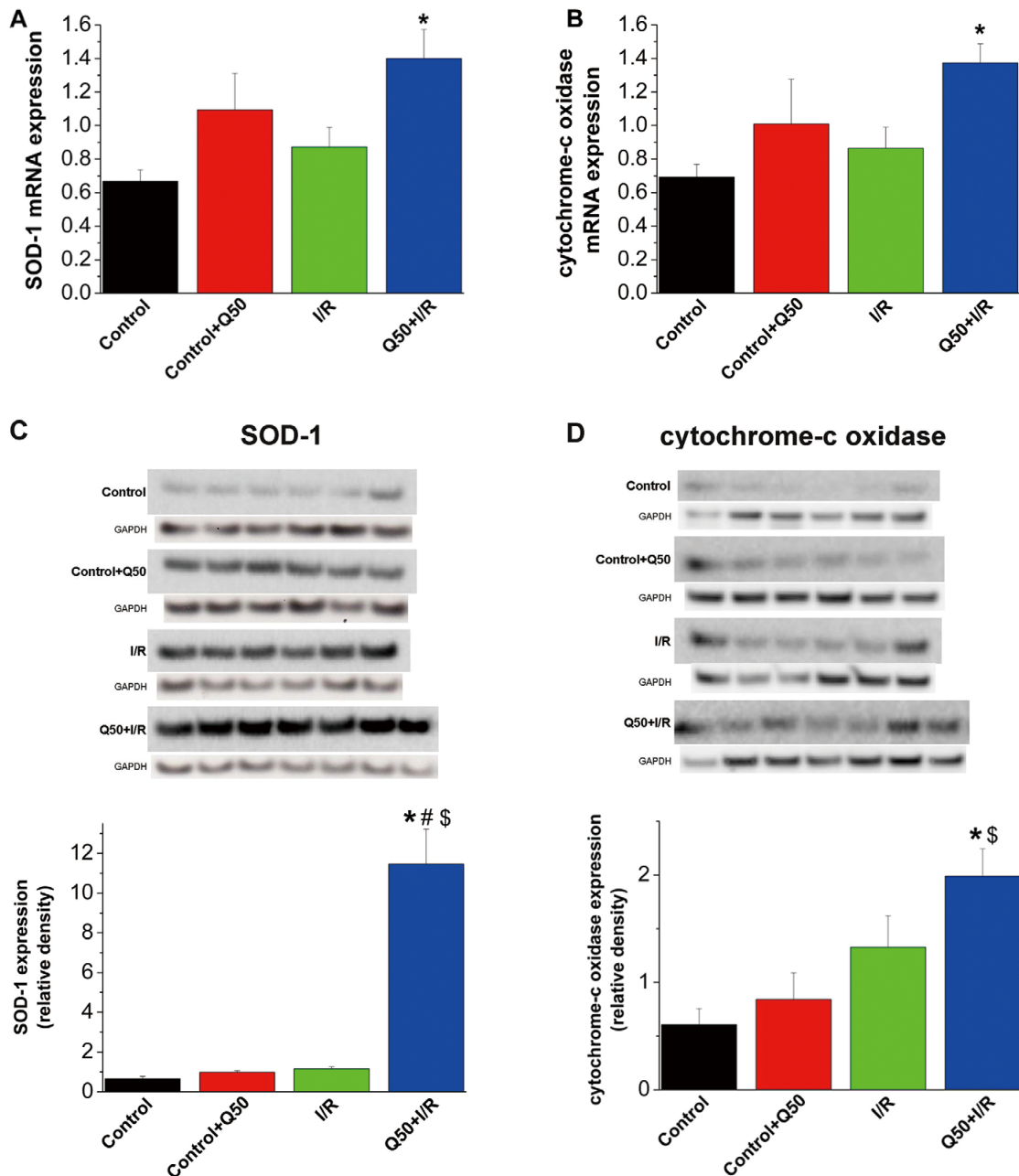


Figure 3. Effect of Q50 on graft gene and protein expression after heart transplantation. (A) Superoxide dismutase (SOD)-1, (B) cytochrome-c oxidase mRNA expression and immunoblot analysis for (C) SOD-1 and (D) cytochrome-c oxidase protein band densities in the myocardium. *P<0.05 vs. control, #P<0.05 vs. control + Q50, \$P<0.05 vs. I/R. I/R, ischemia/reperfusion.

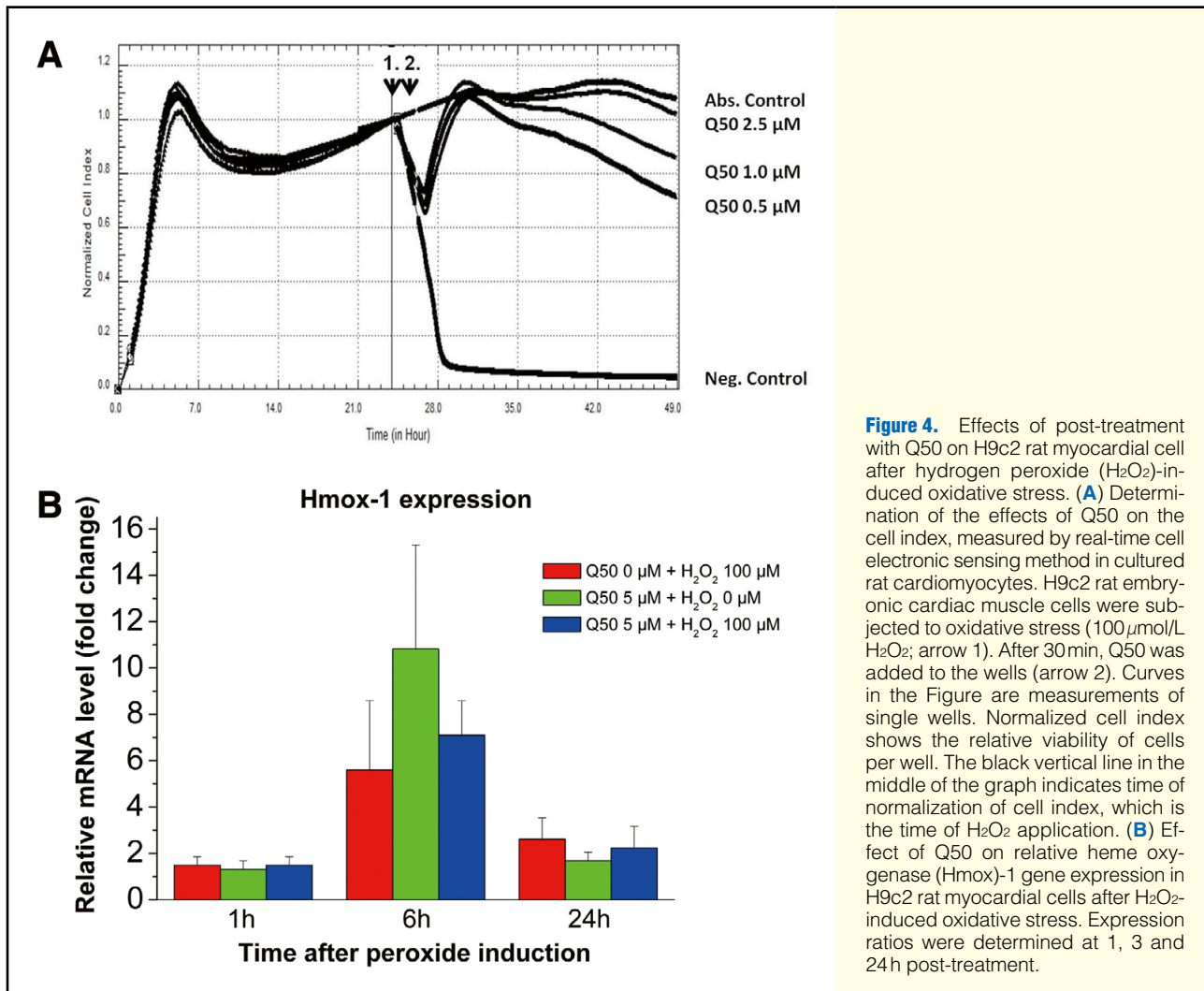


Figure 4. Effects of post-treatment with Q50 on H9c2 rat myocardial cell after hydrogen peroxide (H₂O₂)-induced oxidative stress. **(A)** Determination of the effects of Q50 on the cell index, measured by real-time cell electronic sensing method in cultured rat cardiomyocytes. H9c2 rat embryonic cardiac muscle cells were subjected to oxidative stress (100 μmol/L H₂O₂; arrow 1). After 30 min, Q50 was added to the wells (arrow 2). Curves in the Figure are measurements of single wells. Normalized cell index shows the relative viability of cells per well. The black vertical line in the middle of the graph indicates time of normalization of cell index, which is the time of H₂O₂ application. **(B)** Effect of Q50 on relative heme oxygenase (Hmox)-1 gene expression in H9c2 rat myocardial cells after H₂O₂-induced oxidative stress. Expression ratios were determined at 1, 3 and 24 h post-treatment.

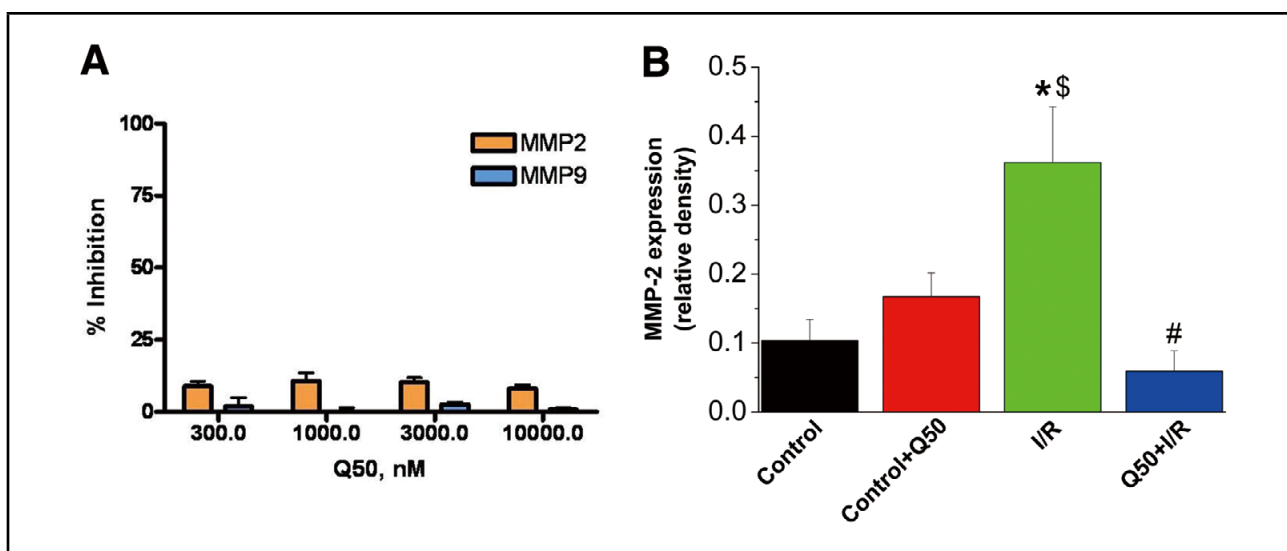


Figure 5. Effect of Q50 on matrix metalloproteinases (MMPs). **(A)** In vitro effects of Q50 on human MMP-2 and MMP-9 enzyme activities. **(B)** Effects of Q50 on myocardial MMP-2 protein expression after heart transplantation. *P<0.05 vs. control, #P<0.05 vs. control+Q50, #P<0.05 vs. I/R. I/R, ischemia/reperfusion.

ment when compared with the control group (Figures 3A,B).

Effect of Q50 on Graft Protein Expression Densitometric analysis of bands for SOD-1 and cytochrome-c oxidase did not show any differences among the control, control-Q50 and I/R groups. However, after heart transplantation, Q50 treatment significantly upregulated the protein expression of SOD-1 when compared with controls and the I/R group (Figure 3C) and increased the cytochrome-c oxidase protein level compared with controls (Figure 3D).

Effect of Q50 Post-Treatment on H9c2 Rat Myocardial Cells After H₂O₂-Induced Oxidative Stress

Cytoprotective Effect of Post-Treatment With Q50 Measured by Real-Time Cell-Microelectronic Sensing Technique Cells were attached and grown overnight and subjected to H₂O₂-induced oxidative stress (Figure 4A). After 30 min, Q50 was added to the wells (Figure 4A) at given concentrations. Normalization of cell index was calculated at the time point of starting H₂O₂ application. Exposure of H9c2 cells to 100 μmol/L H₂O₂ resulted in a rapid decrease of the cell index, whereas the cell index of the absolute control cells that did not receive H₂O₂ treatment remained slightly increased. Post-treatment of Q50 exerted a dramatic dose-dependent cytoprotective effect after H₂O₂ stress: concentrations as low as 0.5 μmol/L maintained the cell index near absolute control levels after the initial 3 h of the experiments and the cell index remained markedly elevated high during the course of the entire experiment.

Effect of Q50 on Relative Heme Oxygenase-1 Gene Expression *Hmox-1* expression ratios were determined at 1, 3 and 24 h post-treatment (Figure 4B). We found pronounced induction (on average, 7-fold) after 3 h. This induction decreased to 2-fold, but remained significantly higher in stressed cells compared with untreated cells. Q50 alone mimicked the effects of H₂O₂ on *Hmox-1* expression; however, when relative mRNA levels were determined in cells treated with Q50 and exposed to H₂O₂, no significant differences were recorded compared with Q50 treatment without applying H₂O₂.

Effect of Q50 on MMPs

Q50 concentrations ranging from 0.3 to 10 μmol/L had no significant effect on the inhibition of either human MMP-2 or MMP-9 enzyme activities (Figure 5A). However, after heart transplantation, the graft protein expression of MMP-2 was significantly increased compared with controls. Q50-treatment of the donor animals at 1 h prior to explantation significantly downregulated graft MMP-2 expression (Figure 5B).

Discussion

I/R injury frequently occurs in a variety of clinical conditions, including MI and heart transplantation. In the present study using rat models of I/R-induced myocardial injury, we showed that administration of Q50, an iron-chelating and zinc-complexing agent, improved cardiac function in vivo and reduced oxidative stress on cardiomyocytes in vitro.

Effects of Q50 Post-Treatment on Cardiac Dysfunction After MI

Using an in vivo experimental model, we studied the cardioprotective effect of Q50 when administered after occlusion before reperfusion to simulate a clinical situation. In the present work, MI was characterized by significantly decreased systolic performance, impaired ventricular relaxation and an increase in end-diastolic volume, indicative of chamber dilata-

tion. End-systolic volume, a marker of ventricular contractility, was also increased in the MI group. We found that administration of Q50 before the onset of reperfusion improved LV systolic function. The major indicator of the transition from reversible to irreversible I/R injury is the release of intracellular cardiac enzymes or markers, such as troponin-T, lactate dehydrogenase, creatine kinase, and aspartate aminotransferase, into the circulation,²¹ reflecting major cellular membrane damage and/or death of cardiomyocytes.²² In the present study, the increased plasma levels of cardiac troponin-T were not reduced by post ischemic administration of Q50. Letienne et al showed that there is a linear relationship between infarct size and plasma levels of biochemical markers.²³ Moreover, Q50 failed to result in a reduction of infarct size after temporary occlusion followed by reperfusion as compared with controls. Even though our study demonstrated improved cardiac function after myocardial ischemia, application of Q50 did not decrease the elevated concentration of cardiac troponin-T or the infarct size, indicating no protective effect on damaged cardiomyocytes. It should be noted that the aforementioned enzyme-biomarker reflects mainly the amount of irreversibly injured myocytes and necrosis, but not the amount of dysfunctional myocytes without irreversible injury. Taken together, these observations support the view that the ability of Q50 to improve cardiac performance may be partially related to this iron-chelating and zinc-complexing agent rescuing cardiomyocytes from the border zones and remote regions of the infarcted heart, improving their function, which in turn leads to an improved global cardiac performance.

Effects of Q50 Pretreatment on Graft Dysfunction After Heart Transplantation

Fast recovery of myocardial function is essential for the success of cardiac transplantation. Therefore, we investigated the effects of Q50 therapy in the early phase (1 h) after heart transplantation. We attempted to simulate clinical conditions encountered during heart transplantation to investigate the potential use of Q50 in enhancing the current protective strategy. We previously described that crystalloid cardioplegia associated with cardiac arrest and reperfusion results in a decline of cardiac function.²⁴ In contrast to the MI rats in which only myocardial contractility was improved by post ischemic administration of Q50, our data showed that treatment of donor rats with Q50 restored both the altered systolic and diastolic LV function after heart transplantation. The different results in these models might be explained by the type of I/R (irreversible vs. reversible) and timing of application (pre- vs. post-ischemic treatment).

Mechanism of Cardioprotective Effects of Q50 Against I/R Injury

One of the most cited mechanisms of reperfusion injury is the generation of free oxygen radicals at the time of reperfusion. These free radicals are superoxide anions, hydroxyl radicals and hydrogen peroxide. Therefore, we studied the effects of Q50 on oxidative stress induced by hydrogen peroxide in cultured cardiomyocytes. By using a cell-microelectronic sensing technique for the screening of cytoprotective compounds,²⁰ we demonstrated pronounced and concentration-dependent cardioprotective effects of Q50 on H₂O₂-treated rat embryonic heart cells in a 30-min post-treatment in vitro model. We found that Q50 induced *Hmox-1* expression with similar kinetics as the H₂O₂ stress in vitro. MMPs are a family of zinc-dependent endopeptidases²⁵ capable of degrading extracellular matrix proteins, and zinc is essential for their proteolytic capacity in this

process. However, inappropriate, prolonged or excessive expression of these enzymes has deleterious consequences. It has been shown that acute release of MMP-2 during reperfusion after ischemia contributes to cardiac mechanical dysfunction,²⁶ and pharmacological inhibition of MMP-2 in rats produced cardioprotection similar to the effect of ischemic preconditioning.²⁷ As a result, MMPs are considered promising drug development targets²⁸ and pharmacological inhibition of MMPs may be a strategy for the treatment of I/R injury. MMPs are generally inhibited by compounds containing reactive zinc-chelating groups.²⁹ In the present study, regardless of the zinc-binding capacity of Q50, this agent did not show enzymatic inhibition of human MMP-2 and MMP-9 in a biochemical assay. However, Q50-treatment of the donor animals 1 h prior to explantation significantly downregulated increased graft MMP-2 expression after heart transplantation. Taken together, we can speculate that in vivo an indirect inhibitory mechanism (ie, binding zinc, which is essential for the catalytic activity of MMPs) might be possible. During I/R injury, the return of oxygen to ischemic tissues is accompanied by an increased production of ROS.³⁰ Iron plays a role in the formation of the free radicals that contribute to oxidative stress, so its chelation makes it unavailable for this. Chelation of ferric iron with deferoxamine, an iron-chelator, has been shown to reduce the production of the hydroxyl radical, thereby reducing myocardial I/R injury.¹⁴ In our model of reversible global myocardial I/R, Q50 treatment resulted in a significant increase in SOD-1 protein expression, a first line defense antioxidant enzyme, indicating both a cytoprotective function and free radical scavenging effect.

In this study, after heart transplantation, comparing with the control group, I/R injury lead to a significant decrease in the high-energy phosphate content. The present data clearly demonstrated that pretreatment with Q50 resulted in a better preservation of the high-energy phosphate pool, primarily by increased myocardial ATP content, resulting in an improved energy status, as expressed by the significant higher energy charge potential. Importantly, the loss of cellular energetic pools, in turn affects myocardial function.³¹ Based on the results of the present study, we propose that Q50 may contribute to better recovery of the cellular ATP and therefore improved myocardial contractility. In pathologic conditions, this iron-chelating and zinc-complexing agent may have promising antioxidant defense mechanisms.

Study Limitations

The present rat model of heterotopic heart transplantation was selected as a suitable model to evaluate I/R injury. The technique includes both reperfusion with blood in a clinically relevant intact animal model, and robust assessment of LV function. This model, however, has certain limitations. In particular, the LV beats in an unloaded condition (ie, the ventricles are perfused via the coronary circulation, but they do not eject), which, on the 1 hand, allows a faster recovery after I/R, but on the other, leads to a time-dependent mechanical deterioration and atrophy. Nevertheless, it has been shown that major deterioration does not occur until at least 24 h after implantation.³² A model of irreversible I/R injury (acute MI) gives rise to many unfavorable changes. Therefore, myocardial gene expression and high-energy phosphate content were only assessed in our model of reversible I/R injury (heart transplantation) to examine the mechanism of action of Q50.

Conclusions

This first in vivo study provides experimental evidence that treatment with an iron-chelating and zinc-complexing agent, Q50, improves cardiac functional recovery of the ischemic/reperfused myocardium in rats. This improvement is independent of reducing infarct size and cardiac enzyme leakage from the infarcted heart. The mechanisms through which Q50 provides cytoprotection may be restoration of myocardial high-energy phosphate levels, upregulation of the antioxidant enzyme SOD-1 protein expression and *Hmox-1* induction. This study suggests that Q50 may be a promising candidate to treat acute MI, myocardial protection during cardiac transplantation and perhaps more generally, open-heart operations involving a period of global ischemia; however, further experimental studies are required. Additionally, because Q50 is a newly developed agent, its subcellular mechanism of action is unknown at present. Therefore, future studies should aim at clarifying possible sites of action of Q50.

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Disclosures

None.

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Supplementary Files

Supplementary File 1

Methods

Please find supplementary file(s);
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