ELSEVIER

Protective Role of 3-Nitro-*N*-Methyl-Salicylamide on Isolated Rat Heart During 4 Hours of Cold Storage and Reperfusion

S. Zhang, J.H. Yang, F. Yu, J. Zhao, P. Jiang, L. Chang, C. Tang, and J. Xu

ABSTRACT

Objective. Cardiac ischemia/reperfusion (I/R) injury, a necessary consequence of transplantation, is probably related to the formation of reactive oxygen species (ROS). The ROS burst within the first moments of reperfusion is associated with injury, continuously generate O_2^- at about 3% to 5% of total O_2 consumption owing to electron leak by mitochondrial oxidoreductases, especially complexes I and III. 3-nitro-*N*-methyl-salicylamide (NNMS) displays inhibitory effects on succinate-cytochrome C reductase, but also reduces effects on creation of O_2^- radical and H_2O_2 by isolated rat mitochondria. Presumably NNMS inhibits electron leakage from the mitochondrial respiratory chain. We investigated effect of NNMS on heart protection after hypothermic ischemia.

Methods. A Langendorff-prepared rat heart model was employed after the heart had been preserved for 4 hours under hypothermic conditions of ischemia with subsequent reperfusion/rewarming for 60 minutes.

Results. The group of hearts treated with NNMS showed increased recovery of heart function compared with a group of mEC. The lactate dehydrogenase (LDH) activity in coronary flow (CF) by hearts treated with NNMS was lower than that with mECs, as was the content of malonedialdehyde (MDA) and conjugated diene (CD).

Conclusions. NNMS improved heart physiology after reperfusion following 4 hours of hypothermic ischemia.

HEART TRANSPLANTATION is the treatment of choice for end-stage disease.[1,2] The most adequate myocardial protection has not been clarified for clinical cardiac transplantation.[3] Currently, heart preservation is limited to 4 to 6 hours of cold ischemic storage.[1,4–6]

The inherent logistics of cadaveric organ donation subject the heart to a period of extracorporeal hypothermic ischemic preservation. Therefore, cardiac ischemia/reperfusion (I/R) injury is a necessary consequence of transplantation. Although, the mechanism of myocardial I/R injury is multifactorial, oxygen free radicals are believed to be major contributors to the reperfusion injury,[7] therefore, possibly benefitted by anti-oxidants, as suggested by Belzer.[8] Diverse sources of oxidants may be injurious to cardiomyocytes.[9–11] Consequently, it has not been possible to engineer a specific anti-oxidant targeted toward preventing myocyte I/R injury. Virtually all major anti-oxidant classes have been examined for this purpose, including endogenous enzymes, xanthine oxidase inhibitors, chain-breaking molecules, iron chelators, and inhibitors of granulocyte function.[12]

Production of reactive oxygen species (ROS) arising during reperfusion considerably damage the structural and functional integrity of transplanted tissues.[13,14] Many studies have used mitochondria as a primary source of ROS in mammalian cells.[15,16] Mitochondria respiration provides ROS as byproducts of electron transfer; about 3% to 5% of the oxygen consumed by mitochondria ends up in H_2O_2 production under normal physiologic conditions. The known precursor of H_2O_2 is the O_2^-, which is generated through a single electron reduction of O_2 by electrons leaking from the substrate side of the respiratory chain.

From the National Laboratory of Biomacromolecule, Center for Molecular Biology, Institute of Biophysics, Chinese Academy of Science (S.Z., J.X.), and the Department of Physiopathology, Institute of Medicine, University of Beijing (J.Y., F.Y., P.J., C.T.), Beijing, China.

Address reprint requests to J. Xu, National Laboratory of Biomacromolecule, Center for Molecular Biology, Institute of Biophysics, Chinese Academy of Science, Beijing 100101, China. E-mail: xujx@sun5.ibp.ac.cn

© 2006 by Elsevier Inc. All rights reserved.
360 Park Avenue South, New York, NY 10010-1710

0041-1345/06/$–see front matter
doi:10.1016/j.transproceed.2006.02.061

Complexes I and III of the respiratory chain are responsible for leaking electrons, thereby generating O_2^-.[17–20] O_2^- and H_2O_2 are the primary sources of ROS. They play prominent roles in diverse pathophysiologic processes, such as aging, neurodegeneration, as well as heart and lung toxicity. It is probable that addition of certain substances to classic cardioplegic and storage solutions may exert beneficial effects against ROS, thereby preserving the functional capacity of the myocardium. The substances may reduce leakage of electrons from complexes I and III of the respiratory chain.

3-Nitro-*N*-methyl salicylamide (NNMS), which was synthesized by our laboratory, was previously shown to mitigate I/R injury in the rat brain.[21] In this study, we observed that NNMS improved recovery of heart function during 4 hours of cold storage and reperfusion using an isolated rat heart model.

MATERIALS AND METHODS

We employed hypothermic conditions to reduce metabolism and thereby limit ischemic damage. Theoretically, lowering preservation temperature by 10°C lowers the cells' metabolic demand by approximately 50%. The generally accepted method of preserving donor heart integrity is arrest with cold (4°C) cardioplegic solution followed by storage in an electrolyte solution immersed in ice. The temperatures attained by this technique are believed to reach 4°C.[2]

Model of Isolated and Perfused Rat Heart

Male Sprague Dawley (SD) rats (weighing 300 to 350 g) were anesthesized using thiopental sodium and injected IV with heparin (500 IU). After the chest was opened using bilateral sternocostal sections, the heart was immediately excised and placed into a cold bath (4°C) containing Krebs-Henseleit buffer (K-HB). It was fixed through the aortic root and left atrium on to perfusion cannulas of a Langendorff apparatus for perfusion at a constant pressure of 60 mm Hg. The K-HB solution had the following composition (mmol/L): NaCl 119.0; $NaHCO_3$ 25.5; KCl 4.3; KH_2PO_4 1.2; $MgSO_4$ 1.2; $CaCl_2$ 2.5; and glucose 11.0. K-HB was used as the perfusion medium. Saturated with 95% O_2 + 5% CO_2 at pH 7.4 at stable temperature of 37°C.

A water-filled latex balloon was inserted into the left ventricle. The balloon was adjusted to a left ventricular end-diastolic pressure (LVEDP) of 6 to 9 mm Hg. We continuously monitored the left ventricle developed pressure (LVDP), its first derivatives ($\pm dp/dt_{max}$), and heart rate (HR). The resultant electrical signals were digitized by MacLab analog to digital converter and recorded by computer. Coronary flow (CF) was measured by timed collection.

After a 20-minute stabilization period, the heart was arrested with 30 mL cardioplegic solution (modified Euro-Collins solution, mECs) at 4°C using a constant pressure of 60 mm Hg. The mEC solution had the following composition (mmol/L): $K_2\ HPO_4 \cdot 3$; H_2O 42.5; $NaHCO_3$ 10.0; KCl 14.0; KH_2PO_4 15.0; $MgSO_4 \cdot 7$; H_2O 5.0; $CaCl_2$ 0.025; and glucose 139.0. Then the heart was disconnected from the circuit, immersed in storage solution, and stored in an icebox for 4 hours. Thereafter, the organ was reperfused at 37°C in Langendorff mode for 60 minutes during which LVEDP, LVDP, $\pm dp/dt_{max}$, and HR were monitored, and CF measured by timed collections. The experiment was divided into 3 groups: group C (n = 8); (2) group mEC (n = 8); and (3) group N+m (n = 8). The group C hearts were not immersed in storage solution. The group mEC hearts were immersed in mEC solution. The group N+m hearts were treated with NNMS (8 μg/mL) added to the mEC solution. To observe whether mEC solution had protective actions, the C group hearts were not immersed in a preservation solution.

Myocardial Water Content

To obtain heart wet weight (WW), the hearts were dried to a constant weight in a 80°C oven for more than 48 hours and reweighed to obtain heart dry weight (DW). We calculated percent myocardial water content (MWC) as follows:

$$MWC(\%) = \left[(WW - DW)/WW\right] * 100\%$$

Malonyldialdehyde Levels

The malonyldialdehyde (MDA) assay method has been described by Askawa.[22] MDA was an estimate of the extent of oxidative damage. In brief, heart biopsy samples (0.3 g) homogenized with 0.7 mL of 0.9% NaCl at 4°C were incubated with 2.25 mL of thiobarbituric acid-reactive substances (TBARS). In 100 mL TBARS were thiobarbituric acid (0.67 g) and trichloroacetic acid (TCA 12 g). Successive procedures included heating to 100°C for 30 minutes followed by cooling and centrifugation (3000 rpm) for 10 minutes. The MDA concentration was determined at 532-nm excitation using a spectrophotometer (721 type made in Shanghai). Results were shown as nanomoles of MDA per mg of protein. The protein content of myocardial tissue was measured by the Bradford[23] method.

Assay of Conjugated Diene

Myocardial conjugated diene (CD) was measured by the method of Corongiu et al.[24] Homogenized heart tissue was extracted; the OD was determined at 233 nm excitation. The results are shown as nanomoles of CD per mg of protein.

Contents of Coronary Flow

The protein content in coronary flow was measured by the Bradford method; myoglobin, (Mb) by the method of Elz et al,[25] lactate dehydrogenase (LDH), by an automated biochemistry instrument.

RESULTS

Effects of NNMS on Functional Parameters

There was no significant difference in the preischemia values of LVEDP. The post-storage values of LVEDP were significantly lower among hearts treated with NNMS than the group C ($P < .05$; (Fig 1C). Mean values of recovery (% prestorage values) of LVDP were 23.19%, 16.71%, and 52.51%, respectively, a significant improvement in LVDP, $+dp/dt_{max}$ and $-dp/dt_{max}$ among hearts treated with NNMS compared with both groups C and mEC both $P < .05$; Fig. 1 A, B, and D). These groups also showed improvements in HR mean value of recovery upon reperfusion compared with groups C and mEC, although the result, did not reach statistical significance. Although NNMS showed 71.28% of the values recorded in the pre-ischemic period; C and mEC groups displayed 65.67% and 59.39%, respectively. There was no significant difference in the mean value of recovery of CF. These results suggested a beneficial role of NNMS in ischemia-reperfusion injury.

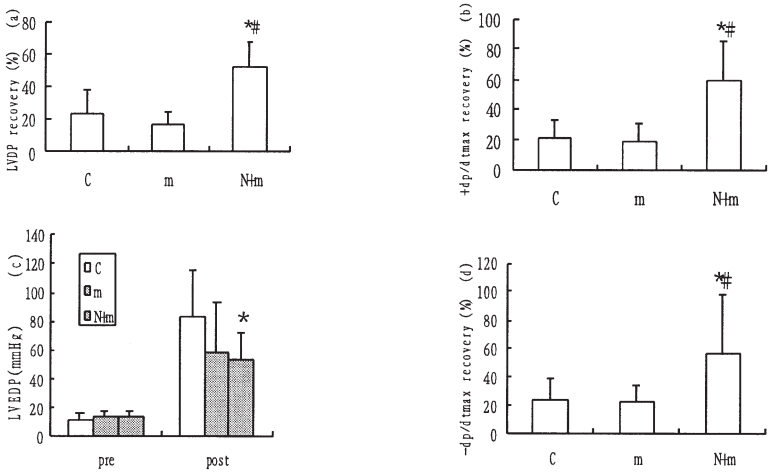

Fig 1. Recovery of cardiac function: mean values of recovery (% prestorage values) were obtained 45 to 60 minutes after reperfusion. *N+m group compared with 'C' group, $P < .05$; #N+m group compared with mEC group, $P < .05$. C, C' group; m, mEC group; N+m, NNMS was added to mEC solution.

Abbreviations: Pre, prestorage; post, post-storage.

Myocardial Water Content Levels Evaluation

There was no significant effect of NNMS treatment on mean values of MWC. The MWC in the NNMS group was 81.006% ± 1.316%, whereas in the C group it was 80.895% ± 2.098%, and in the mEC group, 81.676% ± 1.189%.

Evaluation of Mb, Protein Content, and LDH Activity

There was no significant influence of NNMS on the mean values of Mb content in coronary flow. A significantly lower protein content was in coronary flow of the hearts treated with NNMS compared with group C at 5 and 60 minutes; and significantly reduced compared with both 'C' and mEC groups at 30 minutes (Fig 2 A). There was significant improvement of LDH content in the CF of hearts treated with NNMS compared with groups C and mEC ($P < .05$ and $P < .001$, respectively).

LDH activity in CF in hearts treated with NNMS is shown in Figure 2 B.

Evaluation of MDA and Conjugated Diene

The formation of MDA as an indicator of oxidative injury revealed significant differences in both groups, with the hearts treated with NNMS significantly lower than those stored in only mEC solution: 23.30 ± 7.79 nmol/100 mg Pr in group N+m versus 27.31 ± 6.49 nmol/100 mg Pr in mEC group ($P < .05$; Fig 3 A). The formation of CD is also an indicator of oxidative injury. The mean values of CD that the hearts were treated by NNMS was significant lower than groups C and mEC ($P < .05$; Fig 3 B).

DISCUSSION

Cardiac I/R injury, a necessary consequence of transplantation, is associated with a burst of ROS, which contributes to tissue damage.[26,27] The molecule mechanism of I/R injury has been explained as due to electrons leakage by the respiratory chain mitochondria.[28] The hypoxia of tissue ischemia places the mitochondrial respiratory chain is a high reducing state, which disposes to easy leakage of electrons. Reoxygenation (Reox) of the respiratory chain in a high reducing state may lead to a burst of leaking electrons, generating ROS, which contribute to serious tissue damage. Many studies have demonstrated that mitochondria represent a primary source of ROS in mammalian cells. Two pathways of oxygen consumption are available in mitochondria: One

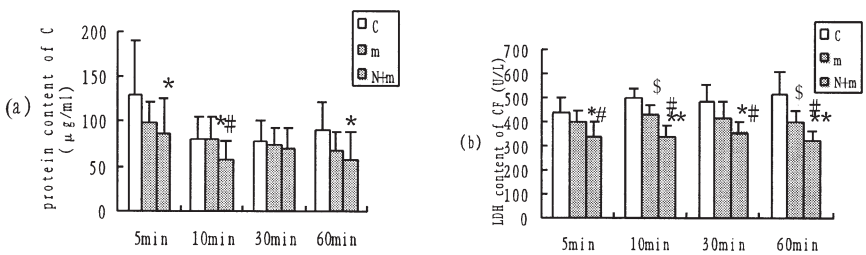

Fig 2. Protein content and LDH activity of coronary flow: coronary flows were obtained at 5, 10, 30, and 60 minutes after reperfusion. *N+m group compared with 'C' group, $P < .05$, **$P < .001$; #N+m group compared with mEC group, $P < .05$. $mEC group compared with 'C' group, $P < .05$. C, 'C' group; m, mEC group; N+m, NNMS was added to mEC solution.

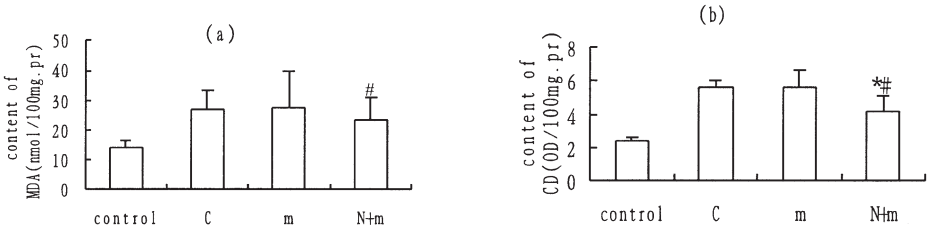

Fig 3. After reperfusion for 60 minutes, content of MDA and CD in heart tissue: *N+m group compared with 'C' group, $P < .05$; #N+m group compared with mEC group, $P < .05$. Control, normal isolation hearts; C, group of the hearts were not immersed in preservation solution; m, mEC group; N+m, NNMS was added to mEC solution.

is O_2 consumption by electrons transferred inside the chain used for ATP synthesis. The other is the O_2 consumed by the electrons leaked out of the chain, which are used in the radical metabolism. The generation of O_2^- radical and H_2O_2 in the substrate side of respiratory chain results from electron leakage of the chain. Complexes I and III of the respiratory chain are responsible for electron leakage and O_2^- radical generation (Fig 4). Hence, it may be beneficial for heart transplantation to use drugs that reduce O_2^- radical generation by electron leakage in the mitochondrial respiratory chain.

NNMS is a methyl salicylate that has undergone nitrification, with addition of salicylamide by methylic acylation (Fig 5). The crystals of NNMS change to yellow raphide. With a melting point of 132°C to 133°C.

We devised a method to measure electron leakage of mitochondria respiratory chain complexes I and III,[29] showing that NNMS reduced generation of O_2^- radical and H_2O_2 by isolated rat myocardial mitochondria. In addition, we found that NNMS had an inhibitory effect on succinate-cytochrome C reductase (Fig 6).

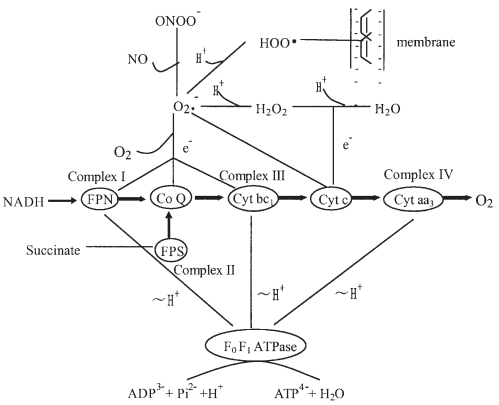

Fig 4. The electron leak-linked radical metabolism (*upper*) and electron transfer-coupled energy metabolism (*lower*) in mitochondria. The cycle linked by arrows (*middle*) is the respiratory chain enzymes. The upper part shows the generation of O_2^- and its four reactive pathways. The lower part shows the coupled ATP synthesis.

Effect of NNMS on Heart Function

Reperfusion of the heart following an ischemic period generates free radicals such as superoxides and hydroxyl radicals. The generated free radicals may induce peroxidation of the intracellular components, such as phospholipids, proteins, and DNA, thus provoking cellular damage. Free radical attack may be one cause of contractile dysfunction of ischemic reperfused hearts.[30,31]

Our results demonstrated that NNMS added to a cardioplegic and storage solution attenuated contractile dysfunction of heart. There was reduced diastolic dysfunction of the heart. Possible mechanisms to explain the effect of NNMS on diastolic dysfunction after reperfusion injury include reduced impairment of calcium homeostasis, specifically calcium sequestration and release by the sarcoplasmic reticulum, a step that is vulnerable to oxygen-derived free radical injury. In addition, free radical disruption of mitochondrial membranes may interfere with adenosine triphosphate production with subsequent ischemic contracture and loss of distensibility.[12]

Differences in MWC between mEC hearts and those treated with NNMS were not significant, although there was a trend toward greater edema in the mEC hearts. David et al[12] suggested the possibility that myocardial edema played a role in diminished distensibility.

Because free radicals generated during I/R periods are considered to peroxidate membrane phospholipids, excessive ROS may cause cell damage by oxidizing DNA, proteins, carbohydrates, and membrane phospholipids. We determined MDA and CD content in the ischemic-reperfused myocardium. There was a reduced myocardial MDA content in hearts treated with NNMS (8 μ g/mL) compared with mEC (23.30% ± 7.79% versus 27.31% ±

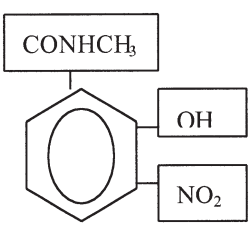

Fig 5. Structure of 3-nitro-N-methyl-salicylamide (NNMS).

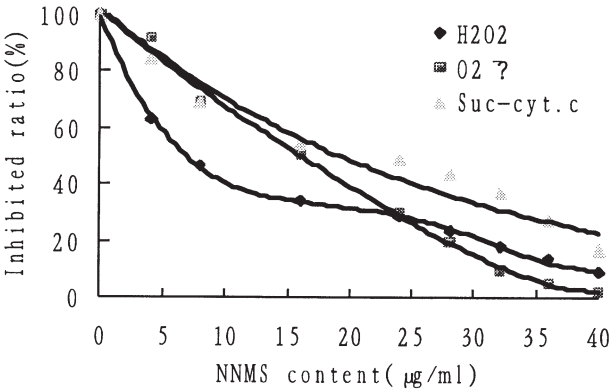

Fig 6. '▲' Inhibition of succinate-cytochrome c reductase by different concentration of NNMS. The reaction system contain 0.2 mmol of phosphate buffer pH 7.4, 1 mmol of succinate, 0.06 mmol of EDTA, 0.05 mmol of cytochrome c. 0.5 mg/mL sub-Mit. Total vilume is 1 mL. '◆' The effect of NNMS on H_2O_2 generation in mitochondria. The method is LDCL assay. Reaction condition: 500 mmol luminol, 2.5 U HRP, 50 mmol phosphate buffer, pH 7.4, 4 mg/mL sub-Mit, 200 mmol NADH. '■' The effect of NNMS on O_2^- generation in mitochondria. The method is LDCL assay. Reaction condition: 250 μmol lucigenin, 60 mmol phosphate buffer, pH 7.4, 4 mg/mL sub-Mit, 200 μmol NADH.

6.49%). The mean value of CD among hearts treated with NNMS was also significant lower than that with mECs ($P < .05$). On the other hand, treated hearts showed improved release of LDH and protein during reperfusion. These effects suggested a contribution of NNMS to protect the heart against free radical attack during ischemia and reperfusion.

Possible mechanisms of a significant influence of NNMS on heart preservation included: (1) prevention of mitochondrial (m) Ca^{2+} overload by reduced ROS generation. ROS generators decrease myofilament Ca^{2+} sensitivity. Although ROS scavengers given after ischemia improve Ca^{2+} sensitivity.[32] (2) Reduced ROS generation preventing opening of mitochondrial permeability transition pores (MPTP). Mitochondrial permeability transition causes mitochondrial to become uncoupled and capable of hydrolyzing rather than synthesizing ATP. Unrestrained, this effect leads to loss of ionic homeostasis and ultimately necrotic cell death. The functional recovery of the Langendorff-perfused heart from ischemia inversely correlated with the extent of pore opening. Inhibition of MPTP provides protection against reperfusion injury.[33] Recent studies have demonstrated that generation of intracellular ROS was enhanced prior to the onset of mitochondrial membrane permeability transition (MPT).[34] Rajesh et al[35] suggested that MPTP inhibition by cyclosporine (CsA) enhanced functional recovery after long term hypothermic heart preservation. Experimental evidence strongly suggests that MPTP opening by lonidamine markedly attenuates the functional recovery of the heart after long-term hypothermic preservation and that lonidamine-treated hearts showed low myocardial ATP levels.[1]

In conclusion, NNMS seemed to be different from other free radical scavengers, since it partially inhibited electron leakage from complex I and III of the mitochondria respiratory chain. If the ROS burst cannot occur at the onset of reperfusion, tissue damage is significant reduced. The present study suggested a new mechanism to eliminate I/R injury due to mitochondrial generation of ROS.

REFERENCES

1. Rajesh KG, Sasaguri S, Tian ZZ, et al: Potential role of mitochondrial permeability transition pore following long-time hypothermic heart preservation. Transplant Proc 34:2645, 2002
2. Amir G, Rubinsky B, Kassif Y, et al: Preservation of myocyte structure and mitochondrial integrity in subzero cryopreservation of mammalian hearts for transplantation using antifreeze proteins-an electron microscopy study. Eur J Cardiothorac Surg 24:292, 2003
3. Llosa JC, Rodriguez Lambert JL, Naya JL, et al: Celsior, a novel cardioplegic solution for arrest and storage in heart transplantation. Transplant Proc 32:2589, 2000
4. Stringham JC, Southard JH, Hegge J, et al: Limitations of heart preservation by cold storage. Transplantation 53:287, 1992
5. Heck CF, Shumway S, Kaye MP: The registry of the International Society of Heart Transplantation: sixth official report. J Heart Transplant 8:271, 1989
6. Hendry PJ, Walley VM, Koshal A, et al: Are temperatures attained by donor hearts during transport too cold? J Thorac Cardiovasc Surg 98:517, 1989
7. Brounwald E, Kloner RA: Myocardial reperfusion: a doubleedged sword? J Clin Invest 76:1713, 1985
8. Belzer FO, Southard JH: Principles of solid-organ preservation by cold storage. Transplantation 45:673, 1988
9. Hearse DJ: Ischemia, reperfusion and the determinants of tissue injury. Cardiovasc Drug Ther 4:767, 1990
10. Kloner RA, Przyklenk K, Whittaker P: Deleterious effects of oxygen radicals in ischemia/reperfusion: resolved and unresolved issues. Circulation 80:1115, 1989
11. Virmani R, Kolodgie FD, Forman MB, et al: Reperfusion injury in the ischemic myocardium. Cardiovasc Pathol 1:117, 1992
12. Rabkin DG, Weinberg AD, Spotnitz HM: Optimizing probucol administration to preserve left ventricular compliance after reperfusion injury in the heterotopic rat heart isograft. J Heart Lung Transplant 22:959, 2003
13. Hearse DJ, Bolli R: Reperfusion induced injury: manifestations, mechanisms and clinical relevance. Cardiovasc Res 26:101, 1992
14. Lojek A, Cerny J, Nemec P, et al: Phagocyte-induced oxidative stress in patients with hemodialysis treatment and organ transplantation. Bio Factors 8:165, 1998
15. Hoffmann S, Spitkovsky D, Radicella JP, et al: Reactive oxygen species derived from the mitochondrial respiratory chain are not responsible for the basal levels of oxidative base modifications observed in nuclear DNA of mammalian cells. Free Radic Biol Med 36:765, 2004
16. Koch OR, Pani G, Borrello S, et al: Oxidative stress and antioxidant defenses in ethanol-induced cell injury. Mol Aspects Med 25:191, 2004
17. Zhang L, Yu L, Yu CA: Generation of superoxide anion by succinate-cyto chrome c reductase from boxine heart mitochondria. J Biol Chem 273:33972, 1998
18. Nohl H, Gille L, Staniek K: The mystery of reactive oxygen species derived from cell respiration. Acta Biochim Pol 51:223, 2004
19. Choksi KB, Boylston WH, Rabek JP, et al: Oxidatively damaged proteins of heart mitochondrial electron transport complexes. Biochim Biophys Acta 1688:95, 2004
20. Riess ML, Camara AKS, Kevin LG, et al: Reduced reactive O_2 species formation and preserved mitochondrial NADH and

[Ca2+] levels during short-term 17°C ischemia in intact hearts. Cardiovasc Res 61:580, 2004

21. Shen M, Xu J, Chen Q: The protective effect of 3-nitro-n-methyl-salicylamide on rat cerebral ischemia reperfusion injury. Journal of Apoplexy and Nervous Diseases 19:17, 2002

22. Askawa T, Matashushita S: Thiobarbituric acid test for detecting lipid peroxide. Lipids 14:401, 1979

23. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248, 1979

24. Corongiu FP, Banni S, Dessi MA: Conjugated dienes detected in tissue lipid extracts by second derivative spectrophotometry. Free Rad Biol Med 7:183, 1989

25. Elz JS, Nayler WG: Quantification of calcium paradox in neonatal rat hearts. Am J Physiol 253:H1358, 1987

26. Granville DJ, Tashakkor B, Takeuchi C, et al: Reduction of ischemia and reperfusion-induced myocardial damage by cytochrome P 450 inhibitors. Proc Natl Acad Sci U S A 101:1321, 2004

27. Becker LB: New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovasc Res 61:461, 2004

28. Jlan-xing XU: The electron leak pathways of respiratory chain and metabolism of superoxide anion in mitochondria and biology significance. Basic Medical Sciences and Clinics 21:389, 2001

29. Zhao Y, Wang Z, Xu J: Effect of cytochrome c on the generation and elimination of O_2^- radical and H_2O_2 in mitochondria. J Biol Chem 278:2356, 2003

30. Koksal C, Suzer O, Bozkurt AK, et al: Comparison of enoximone, amrinone, or levosimendan enriched St.Thomas' hospital cardioplegic solutions used for myocardial preservation in isolated guinea pig hearts. Acta Medica (Hradec Kralove) 45:93, 2002

31. Simpson PJ, Lucchesi BR: Free radicals and myocardial ischemia and reperfusion injury. J Lab Clin Med 110:13, 1987

32. Perez NG, Gao WD, Marban E: Novel myofilament Ca2+ sensitizing property of xanthine oxidase inhibitors. Circ Res 83:423, 1998

33. Halestrap AP, Clarke SJ, Javadov SA: Mitochondrial permeability transition pore opening during myocardial reperfusion—a target for cardioprotection. Cardiovasc Res 61:372, 2004

34. Kanno T, Sato EE, Muranaka S, et al: Oxidative stress underlies the mechanism for Ca (2+)–induced permeability transition of mitochondria. Free Radic Res 38:27, 2004

35. Rajesh KG, Sasaguri S, Ryoko S, et al: Mitochondrial permeability transition—pore inhibition enhances functional recovery after long-time hypothermic heart preservation. Transplantation 76:1314, 2003