Cellular and Molecular Biology [TM] **55**, OL1208-OL1214
DOI 10.1170/137

ISSN 1165-158X
2009 Cell. Mol. Biol.[TM]

EXPRESSION OF THE THREE NITRIC OXIDE SYNTHASE ISOFORMS AND NITRIC OXIDE LEVEL IN THE RAT HEART DURING COLD STORAGE AND BLOOD REPERFUSION

M. DESROIS[1]*, T. CAUS[1,2], C. DALMASSO[1], C. LAN[1], PJ COZZONE[1] AND M. BERNARD[1]

1Centre de Résonance Magnétique Biologique et Médicale (CRMBM), UMR CNRS n°6612, Faculté de Médecine de Marseille, Université de la Méditerranée, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France.
2Department of Cardiac Surgery, Amiens-Picardie University Hospital, Avenue Laënnec-Salouël, 80054 Amiens Cedex 1, France.
* Telephone, +33 (4) 91 25 65 29; Fax, + 33 (4) 91 25 65 39; E -mail, martine.desrois@univmed.fr

Received May 6th, 2009; Accepted September 19th, 2009; Published December 20th, 2009

Abstract – Maintenance of nitric oxide (NO) homeostasis is an important concept for myocardial protection. Here, we have investigated the NO pathway by analysing total nitrate concentration (NOx) and NO synthase (NOS) isoforms expression as well as the myocardial integrity by lactate dehydrogenase and creatine kinase contents in the rat heart graft arrested by CRMBM solution, submitted to 3 hr cold ischemia in the same solution and 24 hr blood reperfusion following heterotopic abdominal heart transplantation. NOx level was similar to baseline value after ischemia and significantly increased after 24 hr reperfusion. NOS isoforms expression was highly modulated after cold ischemia followed by blood reperfusion. Endothelial NOS expression was decreased after ischemia but restored after 24 hr reperfusion. Neuronal NOS expression was drastically decreased after ischemia and 24 hr reperfusion. Inducible NOS protein was present only after 24 hr reperfusion. Cold ischemia induced a severe loss of creatine kinase without any modification after blood reperfusion. In conclusion, we show here that CRMBM solution did not increase NO production during ischemia but induced an enhanced synthesis of NO during reperfusion which may be related to restoration of endothelial NOS expression and/or induction of inducible NOS expression.

Key words: Heart, ischemia-reperfusion, transplantation, nitric oxide, nitric oxide synthase, cellular integrity.

INTRODUCTION

Prolonged heart storage in cardiac transplantation leads to myocardial and endothelial injury that ultimately involves impaired contractile performance and cardiac allograft vasculopathy. Protection of the endothelium and maintenance of nitric oxide (NO) pathway have been recognized as an important concept for myocardial protection (26, 28, 31). Impaired NO homeostasis during ischemia-reperfusion contributes to deleterious effects including myocardial stunning (26). NO originates from L-arginine in a reaction catalyzed by NO synthase (NOS) which exists in three established isoforms: endothelial (eNOS, NOS-3), neuronal (nNOS, NOS-1) and inducible (iNOS, NOS-2). The three NO synthase isoforms are largely distributed in the mammalian myocardium (23) and subserve distinct functions as a consequence of their different cellular and subcellular localization, regulation and coupling to downstream targets. Consequently, it is thought that changes in their expression and activity may have physiological and pathophysiological consequences for long-term outcome of heart graft. In particular, a disregulation of the NO pathway has been reported to play a major role in the development of graft vasculopathy (31).

The CRMBM cardioplegic solution has been developed in our laboratory (2) and optimized to limit endothelial and myocardial dysfunction due to ischemia-reperfusion injury both in *ex vivo* and *in vivo* studies (3, 4, 10, 11). In a preliminary study, we have demonstrated that NOS isoforms play distinctive roles in parallel to changes in myocardial function during short (1 hr) and prolonged (24 hr) reperfusion with the standard CRMBM solution (9). In addition, supplementation of CRMBM solution with L-

Abbreviations: **CRMBM**, Centre de Résonance Magnétique Biologique et Médicale; **NO**, nitric oxide; **NOx**, total nitrate concentration; endothelial (e), neuronal (n), inducible (i) nitric oxide synthase (NOS).

arginine decreased myocardial stunning during 1 hr in vivo reperfusion due to a better preservation of the NO pathway in a heterotopic rat heart transplantation model (4). In the same animal model, we have reported recently that L-arginine-enriched CRMBM solution improved post-ischemic functional recovery, increased high energy compounds and production of NO after 1 hr reperfusion without modification of NOS isoforms, indicating a better myocardial and endothelial preservation of the heart graft than with the commercial solution Celsior (7). To our knowledge, NO production and the three NOS isoforms expression have not been studied during cold storage following cardioplegic arrest and prolonged blood reperfusion. In this context, we have investigated total nitrate concentration (NOx) and the expression of endothelial, neuronal and inducible NOS isoforms in the rat heart graft arrested by optimized CRMBM solution, submitted to 3 hr of cold ischemia in the same solution and 24 hr blood reperfusion following heterotopic abdominal heart transplantation. Myocardial tissue contents of lactate dehydrogenase and creatine kinase were also used as markers of myocardial damage and cellular injury.

MATERIALS AND METHODS

Heterotopic heart transplant model

The experimental model, a modified Ono-Lindsey model, has been described elsewhere (5). Briefly, Lewis inbred male rats weighing 325 to 400 g were used as both donors and recipients. All animals received humane care in compliance with the principle 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 National Institutes of Health (NIH Publication No. 86-23, revised 1996). All investigations in this project were conducted under a license for animal research granted by the French Ministry of Agriculture.

Donors and recipients were anesthetized intraperitoneally with ketamine (80 mg/kg)/xylazine (10 mg/kg) and pentobarbital (40 mg/kg), respectively. Donors intravenously received 1,000 IU sodium heparin. Cardioplegic arrest and preservation of the heart transplants were achieved with CRMBM solution (10). Composition of CRMBM solution is given in Table 1. The CRMBM solution was made extemporaneously in the laboratory. During cold storage, the grafts were kept at 4°C in 100 ml of the respective preservation solution. Total ischemic time was set at 3 hours (hr) for all experiments. For reperfused hearts, heterotopic abdominal heart transplantation was performed as previously described (5), ischemic time included the time necessary to implant the graft into the recipient (30 minutes). At the end of heterotopic abdominal heart transplantation, blood reperfusion was achieved by unclamping the recipient's aorta. The hearts were allowed to recover *in vivo* during 24 hr to evaluate prolonged blood reperfusion.

Table 1. Composition of the CRMBM solution

Components (mM)	CRMBM solution
KCl	2
KH_2PO_4	2
$CaCl_2$	0.25
$MgCl_2$	13
NaOH	120
Lactobionic acid	100
Raffinose	30
Glutamate	20
Mannitol	30
GSH	3
Adenosine	0.5
Allopurinol	1
L-arginine	2
Osmolarity (mOsm/l)	340
pH	7.4

Groups

Hearts were rapidly freeze-clamped with a Wollenberger clamp precooled in liquid nitrogen either after 3 hr of cold ischemia (Ischemia group, n = 5) or after 24 hr blood reperfusion following heterotopic abdominal heart transplantation (24 hr reperfusion group, n = 5-7). For determining baseline values, 6 other hearts were freshly excised from Lewis rats and freeze-clamped (Baseline group, n = 6).

Total nitrate concentration

Tissue total nitrate concentration (NOx) was determined according to the method described by Cross et al (6) using a nitrate/nitrite colorimetric assay kit (Cayman Chemical Compagny, Ann Arbor, MI, USA).

NOS isoforms expression

Endothelial (e), neuronal (n) and inducible (i) NOS isoforms expression was determined by Western blotting using specific primaries antibodies (1/1000, 1/250, 1/500, Transduction Laboratories, USA) as previously described (4, 9).

Lactate dehydrogenase (LDH) and creatine kinase (CK)

Lactate dehydrogenase and creatine kinase activities were measured as previously described (10).

Statistical analyses

Results were expressed as means ± SEM. Comparison between groups was analyzed by one-way ANOVA followed by a Scheffe's post-hoc test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Total nitrate concentration

Tissue total nitrate concentration (NOx) was determined as a measure of NO production (6, 14) and was shown in Figure 1. NOx concentration was similar to baseline value after 3 hr ischemia and significantly increased after 24 hr reperfusion ($p < 0.002$ versus Baseline and $p < 0.005$ versus Ischemia).

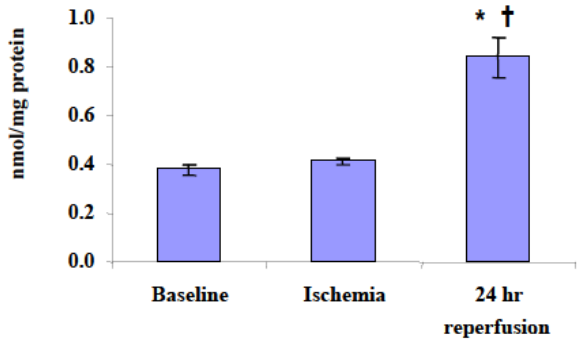

Figure 1. Total nitrate concentration (NOx) in freeze-clamped hearts after ischemia and 24 hr blood reperfusion compared to Baseline. Results are expressed as means ± SEM. Versus Baseline, * $p < 0.002$; versus Ischemia, † $p < 0.005$.

NOS isoforms expression

Endothelial (e), neuronal (n) and inducible (i) NOS isoforms expression were shown in Figures 2A, 2B and 2C respectively. eNOS expression was decreased after cold ischemia ($p < 0.05$ versus Baseline) but restored after 24 hr reperfusion (versus Ischemia, $p < 0.01$). nNOS expression was decreased after cold ischemia and 24 hr reperfusion (versus Baseline, $p < 0.0001$ and $p < 0.01$, respectively). nNOS expression was significantly increased after 24 hr reperfusion compared with Ischemia ($p < 0.02$). By contrast to eNOS and nNOS, iNOS protein was present only after 24 hr reperfusion (versus Baseline, $p < 0.001$; versus Ischemia, $p < 0.001$).

Lactate dehydrogenase (LDH) and creatine kinase (CK)

Myocardial tissue contents in LDH and CK were shown in Table 2. LDH was unchanged in any condition. A significant loss of CK was found after ischemia ($p < 0.001$ versus Baseline) and after 24 hr blood reperfusion ($p < 0.001$ versus Baseline).

DISCUSSION

Protection of endothelium and maintenance of NO pathway are crucial for cardiac transplant outcome (26, 28, 31). Impaired NO homeostasis during ischemia-reperfusion contributes to deleterious effects including myocardial stunning (26). Here, we have focused our study on NO pathway modulation after cold storage following cardioplegic arrest and prolonged reperfusion in a heterotopic rat heart transplantation model. Cardiac ischemia and blood reperfusion may be responsible for either the loss of NOS isoforms or changes in their activity, both conditions possibly leading to coronary dysfunction and myocyte injury but little is known about their modulation during cold storage and blood reperfusion.

The determination of tissue total nitrate concentration (NOx) was used to reflect *in vivo* NO production as previously reported (14, 33). Our baseline value in the rat heart (0.38 ± 0.02 nmol/mg protein) was similar with data obtained by Cross et al (6) in the male mouse heart (32 pmol/mg protein) taking into account the heart weight of each animal respectively and was also similar to the value reported in the rat heart by Xiao et al (32). A good correlation has been found between this measure and the determination of NOS activity *in vitro* by measuring the conversion of L-arginine to L-citrulline (33). Ischemia-reperfusion injury has been reported to be initiated by a massive burst of NO production after onset of ischemia due to increased calcium concentration and activation of eNOS, depleting local L-arginine and/or BH4 concentrations (16, 17, 26). This increased NO production is particularly deleterious because it can induce peroxynitrite synthesis during reperfusion which is known to be involved in myocardial ischemia-reperfusion injury (16, 17). Apart from NOS-derived NO, a non-enzymatic production of NO exists during myocardial ischemia (22, 34). Xanthine oxidase and increased calcium concentration due to intracellular tissue acidosis during ischemia cause an increase in NO production in the heart (12, 27). Interestingly, we did not observe any increase in NO production during cold ischemia with CRMBM solution by contrast to results obtained previously with Celsior solution showing an increased NO production after 3 hr ischemia (8). As a matter of fact, we recently reported lower myocardial and endothelial preservation after 1 hr reflow with Celsior

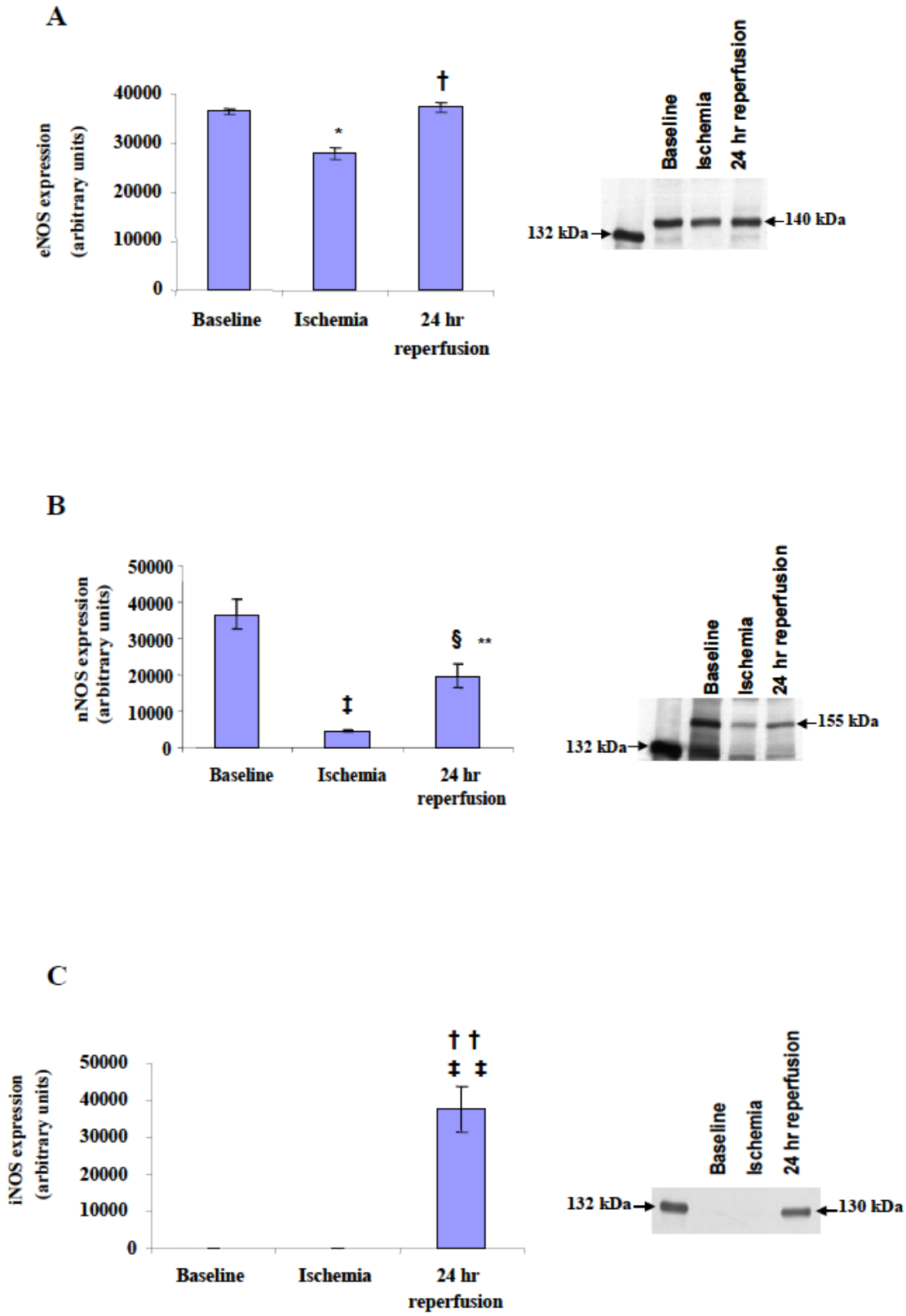

Figure 2. endothelial NOS (eNOS) (A), neuronal NOS (nNOS) (B) and inducible NOS (iNOS) (C) isoforms expression in freeze-clamped hearts after ischemia and 24 hr blood reperfusion compared to Baseline. The bar graphs next to the blots show results from 5-6 rats in each group. Results are expressed as means ± SEM. Versus Baseline, * $p < 0.05$, ‡ $p < 0.0001$, § $p < 0.01$, †† $p < 0.001$. Versus Ischemia, † $p < 0.01$, ** $p < 0.02$, ‡‡ $p < 0.001$.

Table 2. Myocardial tissue contents in lactate dehydrogenase (LDH) and creatine kinase (CK)

	LDH U/mg protein	CK U/mg protein
Baseline	2.84 ± 0.15	10.35 ± 0.57
Ischemia	3.38 ± 0.11	6.18 ± 0.11*
24h reperfusion	2.82 ± 0.19	6.88 ± 0.50†

Versus Baseline, * p = 0.0002, † p = 0.0006.

solution compared with CRMBM solution (7). In addition, increased NO production after 1 hr reperfusion with the CRMBM solution was associated with improved postischemic functional recovery and higher phosphocreatine and ATP concentrations after reflow compared with hearts preserved with Celsior solution (7).

For the first time to our knowledge, we showed here the three NOS isoforms expression after cold ischemia and prolonged reperfusion in a heterotopic rat heart transplantation model. NOS isoforms expression after 24 hr reperfusion was similar to a previous study with the standard CRMBM solution (9), showing that the presence of L-arginine in the optimized CRMBM solution did not modify the expression of NOS isoforms at the stage of reperfusion. A moderate decrease in eNOS expression (23% lower than Baseline value) was observed at the end of ischemia whereas the loss of nNOS expression was more severe (88% lower than Baseline value). It is possible that the loss of eNOS was due to protein degradation by activation of cellular proteases (24) and/or was associated with a combination of pH-dependent denaturation and proteolysis as reported by Giraldez et al (15). However loss of eNOS was limited in the present study, in possible relation with preservation of the protein pool with the CRMBM solution. Interestingly, the loss of eNOS protein was reversed after reperfusion demonstrating restoration of eNOS protein due to new protein synthesis and/or to better preservation of eNOS protein pool. Restoration of eNOS expression after 24 hr blood reperfusion may have a beneficial effect via the cardioprotective effects of this isoform. Effectively, Vejlstrup et al (30) have suggested that a rapid fall in eNOS expression in human myocardial biopsies within the first months following heart transplantation may be responsible for the coronary endothelial dysfunction often seen after human cardiac transplantation. By contrast to eNOS, nNOS protein content was not restored after 24 hr reperfusion. It has been suggested that, in the heart, eNOS normally accounts for most of NO production (1, 15). nNOS appears much less prominent although the exact amount of this isoform in the heart in uncertain (29). Compensatory or deleterious effect of nNOS in ischemic heart remains to be defined (23) and the role of the severe loss of nNOS protein after ischemia-reperfusion injury on graft outcome needs to be clarified in the future. As was observed in this study, late expression of iNOS after reperfusion has been reported previously with varying effects. iNOS expression has been either related to tissue injury (20, 25) or associated to protective effects (21). In conclusion, there are disparate findings regarding the role of iNOS and future studies should be addressed to examine the complex and opposing roles of iNOS in graft transplant outcome.

The CRMBM solution has been optimized to protect myocardial and endothelial function during long-term heart preservation (3, 4, 10, 11). Due to the presence of L-arginine in the solution and its role as a NO precursor, myocardial NO production is an important parameter to evaluate. As we discussed previously in the original paper describing the solution (2), the presence of magnesium and low potassium as well as a moderate calcium content in the CRMBM solution contribute to limit the energy-dependent calcium influx (18) which may reduce the activation of eNOS and nNOS. In addition, anti-oxidants present in the CRMBM solution can modulate reactive oxygen species production and also intracellular Ca^{2+} overload in ischemic-reperfused heart (19) and may protect endothelial function by reducing oxidative stress (13). However, we did not evaluate markers of

oxidative stress in this study. In addition, we reported here that although no significant change in LDH was found in any condition, cold ischemia induced a severe loss of creatine kinase content which was maintained after reperfusion. As it has been reported that ischemia results in activation of cellular proteases (24), the loss of creatine kinase can be due to protein degradation and/or cellular damage. Furthermore, maintenance of CK pool compared to ischemia after reperfusion may be associated to a better protection of cellular integrity due in particular to the low potassium concentration in the CRMBM solution as reported previously (11).

As a limitation of the study, we are aware that the heterotopic rat heart transplantation model is still basically that of an unloaded, perfused heart. It is also important to underline that we have measured the total eNOS, nNOS and iNOS protein levels in cardiac tissues and that the specific location of structures of the heart in which the different types of NOS are specifically upregulated or downregulated cannot be understood.

To the light of the results obtained in the present study, we hypothesize that restoration of eNOS expression and/or induction of iNOS expression during reperfusion may be related to increased NO production. Whether this increased NO production is beneficial for the outcome of heart graft has to be further investigated.

Acknowledgements – This work was supported by CNRS UMR 6612, Assistance Publique-Hôpitaux de Marseille and Association Méditerranéenne pour le Développement des Transplantations (AMDT).

REFERENCES

1. Balligand, J.L., Kobzik, L., Han, X., Kaye, D.M., Belhassen, L., O'Hara, D.S., Kelly, R.A., Smith, T.W., Michel, T., Nitric oxide-dependent parasympathetic signaling is due to activation of constitutive endothelial (type III) nitric oxide synthase in cardiac myocytes. *J. Biol. Chem.* 1995, **270:** 14582-14586.

2. Bernard, M., Caus, T., Sciaky, M., Lan, C., Cozzone, P.J., Optimized cardiac graft preservation: a comparative experimental study using P-31 magnetic resonance spectroscopy and biochemical analyses. *J. Heart Lung Transplant.* 1999, **18:** 572-581.

3. Bernard, M., Robert, K., Caus, T., Desrois, M., Paganelli, F., Cozzone, P.J., Maixent, J.M., Protective effect of a low K+ cardioplegic solution on myocardial Na,K-ATPase activity. *Cell. Mol. Biol. (Noisy-le-grand).* 2004, **50:** 841-844.

4. Caus, T., Desrois, M., Izquierdo, M., Lan, C., LeFur, Y., Confort-Gouny, S., Métras, D., Clarke, K., Cozzone, P.J., Bernard, M., NOS substrate during cardioplegic arrest and cold storage decreases stunning after heart transplantation in a rat model. *J. Heart Lung Transplant.* 2003, **22:** 184-191.

5. Caus, T., Izquierdo, M., Lan, C., Le Fur, Y., Confort-Gouny, S., Cozzone, P.J., Bernard, M., Simultaneous study of metabolism and function following cardioplegic arrest: a novel method of evaluation of the transplanted heart in the rat. *J. Heart Lung Transplant.* 2001, **20:** 575-582.

6. Cross, H.R., Murphy, E., Steenbergen, C., Ca(2+) loading and adrenergic stimulation reveal male/female differences in susceptibility to ischemia-reperfusion injury. *Am. J. Physiol. Heart Circ. Physiol.* 2002, **283:** H481-489.

7. Desrois, M., Caus, T., Belles, P.M., Dalmasso, C., Lan, C., Cozzone, P.J., Bernard, M., Limitation of myocardial and endothelial injury of the rat heart graft after preservation with Centre de Resonance Magnetique Biologique et Medicale (CRMB) solution. *Transpl. Int.* 2008, **21:** 276-283.

8. Desrois, M., Caus, T., Belles, P.M., Dalmasso, C., Lan, C., Cozzone, P.J., Bernard, M., Nitric oxide pathway after long-term cold storage and reperfusion in a heterotopic rat heart transplantation model. *Transplant. Proc.* 2005, **37:** 4553-4555.

9. Desrois, M., Durrans, A., Caus, T., Lan, C., Clarke, K., Cozzone, P.J., Bernard, M., Modulation of the NO pathway during short or prolonged blood reperfusion following ischaemia in a heterotopic rat heart transplantation model. *Transplant. Proc.* 2004, **36:** 1280-1282.

10. Desrois, M., Sciaky, M., Lan, C., Cozzone, P.J., Bernard, M., L-arginine during long-term ischemia: effects on cardiac function, energetic metabolism and endothelial damage. *J. Heart Lung Transplant.* 2000, **19:** 367-376.

11. Desrois, M., Sciaky, M., Lan, C., Cozzone, P.J., Bernard, M., Metabolic and functional effects of low-potassium cardioplegic solutions for long term heart preservation. *Magn. Reson. Mater. Phy. (MAGMA).* 1999, **8:** 77-82.

12. Doel, J.J., Godber, B.L., Eisenthal, R., Harrison, R., Reduction of organic nitrates catalysed by xanthine oxidoreductase under anaerobic conditions. *Biochim. Biophys. Acta.* 2001, **1527:** 81-87.

13. Fenster, B.E., Tsao, P.S., Rockson, S.G., Endothelial dysfunction: clinical strategies for treating oxidant stress. *Am. Heart J.* 2003, **146:** 218-226.

14. Gill, R.M., Braz, J.C., Jin, N., Etgen, G.J., Shen, W., Restoration of impaired endothelium-dependent coronary vasodilation in failing heart: role of eNOS phosphorylation and CGMP/cGK-I signaling. *Am. J. Physiol. Heart Circ. Physiol.* 2007, **292:** H2782-2790.

15. Giraldez, R.R., Panda, A., Xia, Y., Sanders, S.P., Zweier, J.L., Decreased nitric-oxide synthase activity causes impaired endothelium-dependent relaxation in the postischemic heart. *J. Biol. Chem.* 1997, **272:** 21420-21426.

16. Hallstrom, S., Gasser, H., Neumayer, C., Fugl, A., Nanobashvili, J., Jakubowski, A., Huk, I., Schlag, G., Malinski, T., S-nitroso human serum albumin treatment reduces ischemia/reperfusion injury in skeletal muscle via nitric oxide release. *Circulation.* 2002, **105:** 3032-3038.

17. Huk, I., Nanobashvili, J., Neumayer, C., Punz, A., Mueller, M., Afkhampour, K., Mittlboeck, M., Losert, U., Polterauer, P., Roth, E., Patton, S., Malinski, T., L-arginine treatment alters the kinetics of nitric oxide and superoxide release and reduces ischemia/reperfusion injury in skeletal muscle. *Circulation.* 1997, **96:** 667-675.

18. Jimenez, E., del Nido, P., Sarin, M., Nakamura, H., Feinberg, H., Levitsky, S., Effects of low extracellular calcium on cytosolic calcium and ischemic contracture. *J. Surg. Res.* 1990, **49:** 252-255.
19. Kang, S.M., Lim, S., Song, H., Chang, W., Lee, S., Bae, S.M., Chung, J.H., Lee, H., Kim, H.G., Yoon, D.H., Kim, T.W., Jang, Y., Sung, J.M., Chung, N.S., Hwang, K.C., Allopurinol modulates reactive oxygen species generation and Ca2+ overload in ischemia-reperfused heart and hypoxia-reoxygenated cardiomyocytes. *Eur. J. Pharmacol.* 2006, **535:** 212-219.
20. Koch, A., Burgschweiger, A., Herpel, E., Sack, F.U., Schirmacher, P., Szabo, G.B., Karck, M., Schnabel, P.A., Inducible NO synthase expression in endomyocardial biopsies after heart transplantation in relation to the postoperative course. *Eur. J. Cardiothorac. Surg.* 2007, **32:** 639-643.
21. Li, Q., Guo, Y., Tan, W., Stein, A.B., Dawn, B., Wu, W.J., Zhu, X., Lu, X., Xu, X., Siddiqui, T., Tiwari, S., Bolli, R., Gene therapy with iNOS provides long-term protection against myocardial infarction without adverse functional consequences. *Am. J. Physiol. Heart Circ. Physiol.* 2006, **290:** H584-589.
22. Martin, C., Schulz, R., Post, H., Boengler, K., Kelm, M., Kleinbongard, P., Gres, P., Skyschally, A., Konietzka, I., Heusch, G., Microdialysis-based analysis of interstitial NO in situ: NO synthase-independent NO formation during myocardial ischemia. *Cardiovasc. Res.* 2007, **74:** 46-55.
23. Massion, P.B., Pelat, M., Belge, C., Balligand, J.L., Regulation of the mammalian heart function by nitric oxide. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 2005, **142:** 144-150.
24. McCord, J.M., Oxygen-derived free radicals in postischemic tissue injury. *N. Engl. J. Med.* 1985, **312:** 159-163.
25. Pieper, G.M., Roza, A.M., The complex role of iNOS in acutely rejecting cardiac transplants. *Free Radic. Biol. Med.* 2008, **44:** 1536-1552.
26. Podesser, B.K., Hallstrom, S., Nitric oxide homeostasis as a target for drug additives to cardioplegia. *Br. J. Pharmacol.* 2007, **151:** 930-940.
27. Schulz, R., Kelm, M., Heusch, G., Nitric oxide in myocardial ischemia/reperfusion injury. *Cardiovasc. Res.* 2004, **61:** 402-413.
28. Stoica, S.C., Goddard, M., Large, S.R., The endothelium in clinical cardiac transplantation. *Ann. Thorac. Surg.* 2002, **73:** 1002-1008.
29. Ursell, P.C., Mayes, M., Anatomic distribution of nitric oxide synthase in the heart. *Int. J. Cardiol.* 1995, **50:** 217-223.
30. Vejlstrup, N.G., Andersen, C.B., Boesgaard, S., Mortensen, S.A., Aldershvile, J., Temporal changes in myocardial endothelial nitric oxide synthase expression following human heart transplantation. *J. Heart Lung Transplant.* 2002, **21:** 211-216.
31. Weis, M., Cooke, J.P., Cardiac allograft vasculopathy and dysregulation of the NO synthase pathway. *Arterioscler. Thromb. Vasc. Biol.* 2003, **23:** 567-575.
32. Xiao, D.S., Jiang, L., Che, L.L., Lu, L., Nitric oxide and iron metabolism in exercised rat with L-arginine supplementation. *Mol. Cell. Biochem.* 2003, **252:** 65-72.
33. Xuan, Y.T., Tang, X.L., Qiu, Y., Banerjee, S., Takano, H., Han, H., Bolli, R., Biphasic response of cardiac NO synthase isoforms to ischemic preconditioning in conscious rabbits. *Am. J. Physiol. Heart Circ. Physiol.* 2000, **279:** H2360-H2371.
34. Zweier, J.L., Samouilov, A., Kuppusamy, P., Non-enzymatic nitric oxide synthesis in biological systems. *Biochim. Biophys. Acta.* 1999, **1411:** 250-262.