Cardioprotective Effects of Lowering Oxygen Tension After Aortic Unclamping on Cardiopulmonary Bypass During Coronary Artery Bypass Grafting

Takehiro Inoue, MD; Kwansong Ku, MD; Toshio Kaneda, MD;
Zhiwei Zang, MD; Masaki Otaki, MD; Hidetaka Oku, MD

The effect on myocardial reperfusion injury of reducing oxygen tension during reperfusion on cardiopulmonary bypass (CPB) in coronary artery bypass grafting (CABG) was examined at the same time as the influence of diltiazem during CPB was evaluated. A prospective, randomized trial evaluated the hemodynamic and myocardial metabolic recovery in 3 groups of patients undergoing elective CABG; subjects were randomly allocated on the basis of oxygen tension during reperfusion after aortic unclamping: group 1 (n=10) hyperoxic reperfusion (oxygen tension [PO₂]=450–550 mmHg); group 2 (n=10): hyperoxic reperfusion and subsequent continuous infusion of diltiazem (0.5 µg/kg); group 3 (n=10): lowering reperfusate PO₂ (PO₂=200–250 mmHg). Hemodynamic and myocardial metabolic measurements were taken at 6 preset times: before starting the surgical procedure and at 30 min and 3, 9, 21, and 45 h after discontinuation of CPB. The cardiac index in the lowering reperfusate PO₂ group was higher than that of the hyperoxic reperfusion groups at 30 min and 3 h after CPB, and malondialdehyde and troponin-T were significantly lower at 30 min and 3 h, respectively. In comparison with the hyperoxic + diltiazem group, the hemodynamic and myocardial recovery in the lowering reperfusate PO₂ group was improved for about 3 h after CPB. Reduced oxygen tension during reperfusion after aortic unclamping on CPB is more effective against myocardial injury than a calcium antagonist in the short term. It is a convenient and safe management technique that can reduce morbidity and mortality, especially in the severely compromised heart. (*Circ J* 2002; 66: 718–722)

Key Words: Cardiopulmonary bypass; Coronary artery bypass grafting; Oxygen tension

Despite the known reperfusion injury from oxygen-derived free radicals and calcium overload, cardiopulmonary bypass (CPB) during cardiac surgery is routinely hyperoxic!^{1–3} Because the rate of production of reactive oxygen-derived free radicals depends on the oxygen tension (PO₂)^{4,5} reperfusion injury could be reduced by lowering the tension instead of using antioxidants during extracorporeal circulation.⁶ Ihnken et al reported that reducing the oxygen concentration in the CPB circuit to ‘normoxic’ levels reduces the damaging effects of hyperoxia.⁷ On the other hand, calcium antagonists improve myocardial recovery after ischemia–reperfusion^{8,9} although they do have negative inotropic side effects. Of the calcium antagonists, diltiazem has cardioprotective effects in experimental as well as clinical settings.^{10,11}

In the present study, we examined the effects of lowering PO₂ on the hemodynamic and myocardial metabolic recovery for 45 h after discontinuation of CPB. In addition, we evaluated the effect of infusing diltiazem during reperfusion under CPB.

Methods

Patients

The study group comprised 30 consecutive patients undergoing elective coronary artery bypass grafting (CABG) by the same surgeon between January and September 1999. The study was approved by the Institutional Ethics Committee and all patients gave informed consent before inclusion in the study.

Patients were randomly allocated to one of 3 groups based on the PO₂ after aortic unclamping during CPB: group 1 (n=10) hyperoxic reperfusion (PO₂=450–550 mmHg; 490.3±20.5 mmHg); group 2 (n=10): hyperoxic reperfusion (PO₂=450–550 mmHg; 497.5±22.1 mmHg) and continuous infusion of diltiazem (0.5 µg/kg); group 3 (n=10): lowering reperfusate PO₂ (PO₂=200–250 mmHg; 219.7±16.3 mmHg).

Clinical Management

Anesthesia was induced with fentanyl (30–40 µg/kg), diazepam (0.15–0.25 µg/kg) and vecuronium bromide (0.08–0.1 mg/kg), and then maintained with the same drugs. After induction of general anesthesia and systemic application of heparin (300 IU/kg of body weight), CPB (Stockert Instrumente, Munchen, Germany) was initiated in a standard fashion. A hollow fiber membrane oxygenator was used in all cases (Bard HF6000). The extracorporeal circuit was primed with 2,000 ml lactated Ringer’s solution and 5,000 U heparin. Nonpulsatile flows of at least 2.0 L·min⁻¹·m⁻² were used at moderate hypothermia (rectal

(Received March 6, 2001; revised manuscript received April 25, 2002; accepted May 13, 2002)

Department of Cardiovascular Surgery, Kinki University School of Medicine, Osaka, Japan

Mailing address: Takehiro Inoue, MD, Department of Cardiovascular Surgery, Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan. E-mail: rtc-ryo@med.kindai.ac.jp

Table 1 Patient Characteristics in the 3 Groups of Oxygen Tension

	Group		
	Hyperoxic reperfusion (n=10)	Hyperoxia + diltiazem (n=10)	Lowering PO ₂ reperfusion (n=10)
M/F	6/4	5/5	6/4
Age (years)	65.6±1.7	65.8±4.2	65.0±2.2
BSA (m ²)	1.67±0.12	1.59±0.09	1.62±0.19
Body weight (kg)	60.0±2.9	54.5±3.1	59.5±5.1
EF (%)	60.2±11.5	58.5±10.1	62.2±10.8
Coronary disease (2 vessel/3 vessel)	5/5	4/6	4/6
No. of bypass	3.0±0.3	3.3±0.2	3.1±0.3
Aortic clamp time (min)	70.8±10.4	76.6±4.7	68.0±11.8
CPB time (min)	152.1±20.8	159.1±12.0	156.6±9.6

Values are mean±standard error of the mean. BSA, body surface area; EF, ejection fraction; CPB, cardiopulmonary bypass.

Fig 1. Cardiac index (CI) before starting the surgical procedure and at 30 min, 3, 9, 21, and 45 h after discontinuation of cardiopulmonary bypass. Group 1: hyperoxic reperfusion (oxygen tension=450–550 mmHg); Group 2: hyperoxic reperfusion+diltiazem; Group 3: reduced PO₂ reperfusion (oxygen tension=200–250 mmHg). #p<0.05 vs hyperoxic reperfusion group, *p<0.05 vs diltiazem+hyperoxic reperfusion group.

temperature 28–30°C) and did not fall below 2.2 L·min⁻¹·m⁻² during CPB. The coronary sinus was cannulated (Baxter Healthcare Corporation, UT, USA) and antegrade and/or retrograde blood cardioplegia (pH 7.8; K⁺, 20 mmol/L Na⁺, 135 mmol/L; Ca²⁺, 0.5 mmol/L; Mannitol, 21.4 mmol/L) was administered using the same technique in all 3 groups. The actual final composition of the blood cardioplegia was: pH 7.837±0.26; K⁺, 20.9±0.7 mmol/L; Na⁺, 125.8±5.1 mmol/L; Ca²⁺, 0.5±0.1 mmol/L. No significant differences in the hemoglobin concentration (Hb, 6.1±0.2 g/dl) or PO₂ (234.5±10.7 mmHg) of the blood cardioplegia were observed among the 3 groups.

CABG was performed with a standard nonpulsatile CPB technique, with moderate hypothermia and hemodilution (hematocrit, 20–25%). Cardiac arrest was induced with antegrade administration (3 ml/kg) of cold (4°C) Young's solution and followed by infusion of conventional blood cardioplegic solution every 20 min at 10 ml/kg.

After aortic unclamping, the PO₂ on CPB was controlled by changing oxygen delivery and monitored continuously using a blood gas monitor (CDI model 400; 3M Health Care; Tustin, USA). Until the rectal temperature was at least 35°C, the perfusion pressure on CPB was maintained at 50–60 mmHg for almost 20 min after aortic unclamping. Weaning from CPB was done after the patient had been warmed and CPB was discontinued with protamine for

Fig 2. Left ventricular stroke work index (LVSWI) before starting the surgical procedure and at 30 min, 3, 9, 21, and 45 h after discontinuation of cardiopulmonary bypass. Group 1: hyperoxic reperfusion (oxygen tension=450–550 mmHg); Group 2: hyperoxic reperfusion+diltiazem; Group 3: reduced PO₂ reperfusion (oxygen tension=200–250 mmHg).

Fig 3. Systemic vascular resistance index (SVRI) before starting the surgical procedure and at 30 min, 3, 9, 21, and 45 h after discontinuation of cardiopulmonary bypass. Group 1: hyperoxic reperfusion (oxygen tension=450–550 mmHg); Group 2: hyperoxic reperfusion+diltiazem; Group 3: reduced PO₂ reperfusion (oxygen tension=200–250 mmHg).

heparin reversal.

Hemodynamic Study

Before the operation, a radial artery catheter and a Swan-Ganz pulmonary artery catheter (Baxter Healthcare Corp,

Fig4. Dopamine (DOA) and nitroglycerin (NTG) doses before the starting surgical procedure and at 30min, 3, 9, 21, and 45h after discontinuation of cardiopulmonary bypass. Group 1: hyperoxic reperfusion (oxygen tension=450–550mmHg); Group 2: hyperoxic reperfusion+diltiazem; Group 3: reduced PO₂ reperfusion (oxygen tension=200–250mmHg).

Fig6. Troponin-T concentration before starting the surgical procedure and at 30 min, 3, 9, 21, and 45h after discontinuation of cardiopulmonary bypass. Group 1: hyperoxic reperfusion (oxygen tension=450–550mmHg); Group 2: hyperoxic reperfusion+diltiazem; Group 3: reduced PO₂ reperfusion (oxygen tension=200–250mmHg). #p<0.05 vs hyperoxic reperfusion group, *p<0.05 vs diltiazem+hyperoxic reperfusion group.

Edwards Division, Santa Ana, CA, USA) were inserted for continuous monitoring of systemic and pulmonary artery pressures for measuring cardiac output (CO) and pulmonary capillary wedge pressure (PCWP). Hemodynamic measurements were taken at 6 preset times: before starting the surgical procedure and at 30 min and 3, 9, 21, and 45 h after discontinuation of CPB. Arterial pressure (AP) and central venous pressure (CVP) were measured continuously. CO was determined by the thermodilution technique and PCWP was measured immediately before each CO measurement. Systemic vascular resistance index (SVRI) was calculated with the formula: $\text{SVRI} (\text{dyne}\cdot\text{s}/\text{cm}^5) = 80 \times \text{mean AP} ((\text{MAP}) - \text{CVP}) / \text{cardiac index (CI)}$. The left ventricular stroke work index (LVSWI) was calculated according to the formula: $\text{LVSWI} (\text{gm}\cdot\text{m}^{-2}\cdot\text{beat}^{-1}) = 13.6 \times (\text{CI}/\text{heart rate (HR)}) \times (\text{MAP} - \text{PCWP})$. Postoperative preload and afterload were optimized by assistants who were unaware of the cardioplegic technique used.

Fig5. Creatine phosphokinase-MB (CPK-MB) concentration before starting the surgical procedure and at 30min, 3, 9, 21, and 45h after discontinuation of cardiopulmonary bypass. Group 1: hyperoxic reperfusion (oxygen tension=450–550mmHg); Group 2: hyperoxic reperfusion+diltiazem; Group 3: reduced PO₂ reperfusion (oxygen tension=200–250mmHg).

Fig7. Malondialdehyde (MDA) concentration before starting the surgical procedure and at 30 min, 3, 9, 21, and 45h after discontinuation of cardiopulmonary bypass. Group 1: hyperoxic reperfusion (oxygen tension=450–550mmHg); Group 2: hyperoxic reperfusion+diltiazem; Group 3: reduced PO₂ reperfusion (oxygen tension=200–250mmHg). #p<0.05 vs hyperoxic reperfusion group, *p<0.05 vs diltiazem+hyperoxic reperfusion group.

Blood Samples and Measurements

Arterial and venous samples were assayed to estimate the myocardial release of creatine kinase (CK), lactate, lactate dehydrogenase (LDH-1, LDH-2), troponin-T and malondialdehyde at 30 min and 3, 9, 21, and 45 h after discontinuation of CPB. The blood concentration of CK was measured by a UV method, lactate and LDH by enzymatic methods, and troponin-T by an enzyme immunoassay method.

Statistical Analysis

Group data are expressed as mean $\pm$ standard deviation. A statistical analysis was performed using a one-way ANOVA. When significant differences were detected, pairwise comparisons were made using the Scheffe F test. Statistical significance was assumed at $p < 0.05$.

Results

The clinical characteristics of patients are summarized in

Table 1; there were no significant differences between the groups.

Pre- and Postoperative Cardiac Function

Before the surgical procedure began, there were no significant differences in the hemodynamics of the 3 groups nor did they differ in preoperative ejection fraction on left ventricular angiography. Additionally, all groups received the same preoperative medications, such as nitrate and anticoagulants. At 30 min and 3 h after discontinuation of CPB, the CI in group 3 was significantly higher than in groups 1 and 2, but at 9 h after discontinuation of CPB, no significant difference was observed among the 3 groups. No significant difference in CI was found between groups 1 and 2 before or after discontinuation of CPB. The absolute values are shown in Fig 1.

At 30 min and 3 h after discontinuation of CPB, the LVSWI in group 3 tended to be higher than in groups 1 and 2 (Fig 2). At 30 min and 3, 21, and 9 h after discontinuation of CPB, the SVRI in group 3 tended to be lower than in groups 1 and 2 (Fig 3).

No significant differences in systolic aortic pressure, PCWP, and CVP were found among the 3 groups.

Pre- and Postoperative Doses of Infused Dopamine (DOA) and Nitroglycerin (NTG)

The doses of DOA before the surgical procedure and after discontinuation of CPB did not differ significantly nor was there a significant difference in the dose of NTG among the groups (Fig 4).

Pre- and Postoperative Biochemistry

After discontinuation of CPB, there were no significant differences in the CK, LDH-1, LDH-2 and lactate concentrations among the 3 groups. Although there was not a significant difference in the CK-MB concentration among the 3 groups, it tended to be lower in group 3 than in groups 1 and 2 (Fig 5). The troponin-T concentration in group 3 was significantly lower than in groups 1 and 2 at 3 h after discontinuation of CPB (Fig 6), and was also significantly lower than in groups 1 and 2 at 9 h and 21 h, respectively, after discontinuation of CPB.

The malondialdehyde concentration in group 3 was significantly lower than in groups 1 and 2 at 30 min after discontinuation of CPB (Fig 7).

Discussion

The concept of reperfusion injury remains controversial with several proposed mechanisms, but it is mainly oxygen-derived free radicals and calcium overload that play an important role in the development of left ventricular dysfunction, stunning, reperfusion arrhythmias, vascular damage, and endothelial dysfunction.^{2,3,12,13}

During adult cardiac surgery, CPB is commonly hyperoxic fashion and CPB can itself also lead to the production of oxygen-derived free radicals.^{1,14} The cell protects itself by ubiquitous endogenous free radical scavengers, which may be depleted by ischemia and additionally overwhelmed by the burst of free radical production that occurs with the onset of reoxygenation or hyperoxia after reperfusion with aortic unclamping during CPB.¹⁵ Prasad et al have shown that superoxide dismutase and catalase, which are scavengers of superoxide anion and hydrogen peroxide, respectively, are effective in preventing postpump cardiac

dysfunction,¹⁴ and Ku et al have reported that the hydroxyl radical scavenger, nicaraven, improves the recovery of cardiac function following preservation and reperfusion.¹⁶

The present study showed that lowering the reperfusion PO₂ after aortic unclamping on CPB reduced oxidative myocardial damage and the effects on the hemodynamics and myocardial enzyme release persisted for approximately 3 h after discontinuation of CPB. In fact, the CI in the lowering reperfusion PO₂ group was higher than in the hyperoxic reperfusion groups at 30 min and 3 h after discontinuation of CPB. Additionally, malondialdehyde and troponin-T concentrations were significantly lower at 30 min and 3 h, respectively, after CPB in the lowering reperfusion PO₂ group compared with the hyperoxic reperfusion groups.

It has been shown that the myocardial damage that occurs with reoxygenation depends on the oxygen tension, and that lowering oxygen tension will result in reduced creatine kinase release.⁶ In the present study, the CK-MB concentration in the lowering reperfusion PO₂ group tended to be lower after discontinuation of CPB than in the hyperoxic reperfusion group. Lowering the PO₂ reperfusion during CPB may avoid the deleterious reoxygenation process that leads to a burst of free radicals.^{17,18} Ihnken reports that normoxic CPB in the hypoxic immature heart reduces reoxygenation injury and improves myocardial function,¹⁸ and the benefits of normoxic CPB have been seen in adult normoxic hearts also. We reported recently that maintaining a more physiologic PO₂ during reperfusion following ischemia attenuates reperfusion injury.¹⁹

The mechanism of reperfusion injury is, in part, attributed to calcium overload^{2,3} and there is experimental evidence of calcium pump dysfunction in reperfusion injury.^{3,20} Although calcium antagonists were initially designed as antianginal agents, they were found to preserve the ischemic myocardium^{8,9} and have been tested extensively in regard to myocardial protection. Diltiazem is a calcium antagonist that has a potent cardioprotective effect during reperfusion at low doses and in a dose-dependent fashion.^{8,10} However, Dagenais et al reported that diltiazem is less effective in improving both coronary vasodilatation and left ventricular hemodynamics.²¹ Clinical studies have investigated the beneficial effects of adding calcium antagonists to cardioplegic solutions to optimize cardiac preservation during open heart surgery and Christakis et al in particular found reduced ischemic injury after the addition of diltiazem to the cardioplegia during elective coronary bypass.²²

In addition, calcium antagonists have been implicated as antioxidants. Noronhata-Dutra et al reported that calcium antagonist pretreatment prevented in vivo generation of free radicals by adrenaline²³ and Obata et al demonstrated that diltiazem suppressed hydroxyl free radical generation by ischemic reperfusion in the rat heart.²⁴ Therefore, diltiazem may suppress free radical generation and thus prevent the myocardial ischemic injury associated with generation of free radicals, directly or indirectly. Diltiazem acts during ischemic reperfusion as a calcium channel blocker predominantly and decreases calcium overload, but the precise mechanism is unclear and requires further research.

In the present clinical study, the patients were divided into 3 subgroups to determine whether, in comparison with diltiazem infusion, lowering the oxygen tension would reduce the reperfusion injury in the early period after cardiac surgery. The diltiazem dosage conformed to previ-

ous studies,^{10,22} but we did not find a significant difference in CI between the hyperoxic PO₂+diltiazem group and the hyperoxic PO₂ group. Further studies are needed in this regard. Additionally, the CI in the lowering reperfusate PO₂ group was higher than in the hyperoxic PO₂+diltiazem group at 30 min and 3 h after discontinuation of CPB. Likewise, the malondialdehyde concentration in the lowering reperfusate PO₂ group was less than in the hyperoxic PO₂+diltiazem group at 30 min after discontinuation of CPB. Reduction of oxygen tension may have more effect on postpump myocardial performance than calcium antagonists for several hours after CPB and because it is easy to control oxygen delivery on CPB, this technique is convenient and safe.

Study Limitations

We documented short-term results, but for complete understanding of the oxidative reperfusion injury in adult heart surgery, the long-term effects of oxidative reperfusion damage have to be studied. In the present study, the patients were divided into 2 groups according to the PO₂ level, but for more detailed data, there should be several groups with other oxygen tension levels. Additionally, there is a potential discrepancy in the relationship between the oxygen concentration achieved by lowering PO₂ on CPB and the cardioprotective effects, including the limitation of the lipid peroxidation. It may be a limitation of the present clinical study and further study, for example using free radical scavenging drugs, is needed.

Conclusion

Lowering the reperfusate PO₂ after aortic unclamping on CPB was more effective in reducing reperfusion injury than a calcium antagonist in the short term, and in the severely compromised heart, lowering the PO₂ on CPB may potentially reduce morbidity and mortality. Although CPB is commonly hyperoxic, the present results support our hypothesis that a reduced oxygen tension during reperfusion after aortic unclamping on CPB can reduce oxidative myocardial damage.

References

1. Cavarocchi NC, England MD, Schaff H, Russo P, Orszulak TA, Schnell WA, et al. Oxygen free radical generation during cardiopulmonary bypass: Correlation with complement activation. *Circulation* 1986; **74**(Suppl): III130–III133.
2. Miyamae M, Camacho A, Weiner MW. Attenuation of postischemic reperfusion injury is related to prevention of [Ca²⁺]_i overload in rat hearts. *Am J Physiol* 1996; **271**: H2145–H2153.
3. Mochizuki S, Jiang C. Na⁺/Ca²⁺ exchanger and myocardial ischemia/reperfusion. *Jpn Heart J* 1998; **39**: 707–714.
4. Littauer A, De Groot H. Release of reactive oxygen by hepatocytes on reoxygenation: Three phases and role of mitochondria. *Am J Physiol* 1992; **262**: G1015–G1020.
5. Ihnken K, Morita K, Buckberg GD. Studies of hypoxemic/reoxygenation injury: With aortic clamping. XI. Cardiac advantages of normoxemic versus hyperoxemic management during cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1995; **110**: 1255–1264.
6. Hearse DJ, Humphrey SM, Bullock GR. The oxygen paradox and the calcium paradox: Two facets of the same problem. *J Mol Cell Cardiol* 1987; **10**: 641–668.
7. Ihnken K, Winkler A, Schlensak C, Sarai K, Neidhart G, Unkelbach U, et al. Normoxic cardiopulmonary bypass reduces oxidative myocardial damage and nitric oxide during cardiac operations in the adult. *J Thorac Cardiovasc Surg* 1998; **116**: 327–334.
8. Becker BF, Mobert J. Low-dose calcium antagonists reduce energy demand and cellular damage of isolated hearts during both ischemia and reperfusion. *Naunyn-Schmiedeberg Arch Pharmacol* 1999; **360**: 287–294.
9. Seitelberger R, Zwolfer W, Huber S, Schwarzacher S, Binder TM, Peschl F, et al. Nifedipine reduces the incidence of myocardial infarction and transient ischemia in patients undergoing coronary bypass grafting. *Circulation* 1991; **83**: 460–468.
10. Herzog WR, Vogel RA, Schlossberg ML, Edenbaum LR, Scott HJ, Serebruany VL. Short-term low dose intracoronary diltiazem administered at the onset of reperfusion reduces myocardial infarct size. *Int J Cardiol* 1997; **59**: 21–27.
11. Gambelli G, Donati R, Mezzanotte R, Pozzar F, Cesario AS, Schiavo B, et al. Improvement of postinfarct left myocardial contractile function after administration of diltiazem. *G Ital Cardiol* 1998; **28**: 249–258.
12. Opie LH. Reperfusion injury and its pharmacologic modification. *Circulation* 1989; **80**: 1049–1062.
13. Kukreja RC, Janin Y. Reperfusion injury: Basic concepts and protection strategies. *J Thromb Thrombolysis* 1997; **4**: 7–24.
14. Prasad K, Chan WP, Bharadwaj B. Superoxide dismutase and catalase in protection of cardiopulmonary bypass-induced cardiac dysfunction and cellular injury. *Can J Cardiol* 1996; **12**: 1083–1091.
15. Dhaliwai H, Kirshenbaum LA, Randhawa AK, Singal PK. Correlation between antioxidant changes during hypoxia and recovery on reoxygenation. *Am J Physiol* 1991; **261**: H632–H638.
16. Ku K, Kin S, Hashimoto M, Saitoh Y, Nosawa S, Iwasaki S, et al. The role of a hydroxyl radical scavenger (nicaraven) in recovery of cardiac function following preservation and reperfusion. *Transplantation* 1996; **62**: 1090–1095.
17. Ihnken K, Morita K, Buckberg GD, Winkelmann B, Beyersdorf F, Sherman MP. Reduced oxygen tension during cardiopulmonary bypass limits myocardial damage in acute hypoxic immature piglet hearts. *Eur J Cardiothorac Surg* 1996; **10**: 1127–1134; Discussion 1135.
18. Ihnken K. Myocardial protection in hypoxic immature hearts. *Thorac Cardiovasc Surg* 2000; **48**: 46–54.
19. Kaneda T, Ku K, Inoue T, Onoe M, Oku H. Postischemic reperfusion injury can be attenuated oxygen tension control. *Jpn Circ J* 2001; **65**: 213–218.
20. Smart SC, Sagar KB, el Schultz J, Warltier DC, Jones LR. Injury to the Ca²⁺ ATPase of the sarcoplasmic reticulum in anesthetized dogs contributes to myocardial reperfusion injury. *Cardiovasc Res* 1997; **36**: 174–184.
21. Dagenais F, Cartier R, Hollmann C, Buluran J. Calcium-channel blockers preserve coronary endothelial reactivity after ischemia-reperfusion. *Ann Thorac Surg* 1997; **63**: 1050–1056.
22. Christakis GT, Fremes SE, Weisel RD, Tittley JG, Mickel DA, Ivanov J, et al. Diltiazem cardioplegia: A balance of risk and benefits. *J Thorac Cardiovasc Surg* 1986; **91**: 647–661.
23. Noronha-Dutra A, Steen-Dutra EM, Wooif N. An antioxidant role for calcium antagonists in the prevention of adrenaline mediated myocardial and endothelial damage. *Br Heart J* 1991; **65**: 322–325.
24. Obata T, Yamanaka Y. Protective effect of diltiazem on myocardial ischemic injury associated with OH generation. *Comp Biochem Physiol A Physiol* 1997; **117**: 257–261.