Metabolic and oxidative effects of sevoflurane and propofol in children undergoing surgery for congenital heart disease[1]

Efeitos metabólicos e oxidativos do sevoflurano e do propofol em crianças portadoras de cardiopatia congênita submetidas à cirurgia

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ABSTRACT
PURPOSE: To evaluate the metabolic and oxidative effects of sevoflurane and propofol in children undergoing surgery for correction of congenital heart disease.
METHODS: Twenty children with acyanotic congenital heart disease, scheduled for elective cardiac surgery with cardiopulmonary bypass, age range 1 day to 14 years were randomly assigned to 2 groups: Group GP, programmed to receive total intravenous anesthesia with propofol and group GS scheduled to use balanced anesthesia with sevoflurane. Exclusion criteria were cyanotic heart disease or complex, association with other malformations, severe systemic diseases, infection or children undergoing treatment and palliative or emergency surgery. Blood samples were collected at three different time-points: T0, after radial artery cannulation, T1, 30 minutes after cardiopulmonary bypass (CPB) launch and T2, at the end of procedure. Parameters analyzed included thiobarbituric acid-reactive substance (TBARS), glutathione (GLN), lactate and pyruvate plasmatic concentrations.
RESULTS: TBARS, GSH, lactate and pyruvate concentrations did not change significantly by Friedman´s test. Lactate/pyruvate ratio (L/P) was >10 in both groups. There was a moderate Pearson correlation for TBARS, in T1 (r=0.50; p=0.13) e T2 (r=0.51;p=0.12). Pearson correlation was high between groups during CPB (T1) for lactate (r=0.68; p=0.02), pyruvate (r=0.75; p=0.01) and L/P ratio (r=0.83; p=0.003).
CONCLUSION: Anesthetic techniques investigated in this study showed a similar pattern, with no increase in metabolic substrates and oxidative stress during surgical correction of congenital heart defects in non-cyanotic children.
Keywords: Oxidative Stress. Metabolism. Cardiac Surgical Procedures. Anesthetics, Inhalation. Anesthetics, Intravenous.

RESUMO
OBJETIVO: Avaliar os efeitos metabólicos e oxidativos da anestesia com sevoflurano ou propofol em crianças portadoras de cardiopatia congênita, submetidas à cirurgia eletiva.
MÉTODOS: Vinte crianças com cardiopatia congênita acianótica, agendadas para a cirurgia cardíaca eletiva com circulação extracorpórea (CEC), idades 1 dia-14 anos, foram distribuídas aleatoriamente em dois grupos: Grupo GP (anestesia venosa total com propofol) e grupo GS (anestesia balanceada com sevoflurano). Critérios de exclusão foram: doença cardíaca cianótica ou complexa, associação com outras malformações, doença sistêmica grave, infecção ou crianças submetidas a tratamento e cuidados paliativos ou cirurgia de emergência. Amostras de sangue foram coletadas em três horários diferentes: T0, após a canulação da artéria radial, T1, 30 minutos após o início da CEC e T2, no final do procedimento. Parâmetros analisados: substâncias reativas ao ácido tiobarbitúrico (TBARS), glutationa (GLN), lactato e piruvato.
RESULTADOS: As concentrações de TBARS, GSH, lactato e piruvato não foram diferentes (teste de Friedman). A razão Lactato/piruvato (L/P) foi >10 em ambos os grupos. Houve uma correlação de Pearson moderada no TBARS, em T1 (r = 0,50, p = 0,13) e T2 (r = 0,51, p = 0,12). A correlação de Pearson foi alta entre os grupos durante a CEC (T1) para lactato (r=0,68, p=0,02), piruvato (r=0,75, p=0,01) e relação L/P (r =0,83, p=0,003).
CONCLUSÃO: As técnicas anestésicas investigadas mostraram um padrão semelhante, sem aumento de substratos metabólicos ou do estresse oxidativo durante a correção cirúrgica de cardiopatias congênitas em crianças acianóticas.
Descritores: Estresse Oxidativo. Metabolismo. Procedimentos Cirúrgicos Cardíacos. Anestésicos Inalatórios. Anestésicos Intravenosos.

Introduction

Greater knowledge of congenital diseases linked to the development of new surgical techniques for correction of congenital defects has been responsible for the significant reduction of morbidity and mortality in pediatric patients[1]. However, despite all its achievements, the morbidity and mortality found in some centers, particularly in relation to surgery performed during the neonatal period, are not negligible. Moreover, many of congenital heart disease considered inoperable in the past, are currently treated surgically[2].

The goal of anesthetic management of children with congenital heart disease is to ensure an ongoing intra-and postoperative cardiovascular stability with attenuation of the stress response and nociceptive stimulation. Congenital heart surgery (CHS) in children can be challenging as these procedures generate a systemic inflammatory reaction and endocrine-metabolic stress responses. Hyperglycemia is common during and after CHS[3,4], and is associated with increased morbidity and mortality[5,6].

Many differences between children and adults affect the response to cardiopulmonary bypass, and should be considered in strategies during cardiac surgery. The problems faced in these complex patients and procedures require that infant and neonatal cardiac surgery be performed in specialized centers with a multidisciplinary approach and specialized personnel[7].

In designing the anesthetic plan for children undergoing cardiac surgery, the choice of the anesthetic agent may often appear irrelevant for some professionals. The general belief is that the best results would be related to the use of a technique or a drug with which the anesthesiologist is familiar. Anesthetics propofol and sevoflurane are used in modern anesthetic practice, providing adequate anesthesia when inhaled or used intravenously during surgical procedures. Last decade studies indicate that volatile anesthetics have cardioprotective effects when administered before an ischemic event, so-called anesthetic induced preconditioning[8,9]. The intravenous anesthetic agent propofol has also been reported to have cardioprotective effects[10,11].

The definition of reliable markers for monitoring tissue perfusion in pediatric cardiac surgery has been a constant object of study, not only to predict morbidity and mortality, but also to maintain a cost-effective treatment. The quantification of oxidative stress is difficult to be translated through methods and markers that have wide clinical use. Whereas reactive oxygen species (ROS) have very short half-life, their evaluation is done by detection of products resulting from lipid peroxidation (thiobarbituric acid reactive substances - TBARS) and products capable of inactivating these ROS such as glutathione[12]. Blood serum lactate is probably the best biochemical predictor of adverse events in children undergoing cardiac surgery[13]. Hiperlactacemia during CPB in children with congenital heart disease, and its value as an early indicator of morbidity and mortality has been described[14,15].

Whereas the information available in the literature suggest that both the sevoflurane and propofol seem to have cardioprotective effect in children undergoing cardiac surgery, this study aimed to evaluate the influence of anesthetic technique on the occurrence of metabolic effects of surgical stress and inflammatory response associated with cardiopulmonary bypass (CPB), and on ischemia-reperfusion injuries in pediatric cardiac surgery.

Methods

This prospective, randomized, comparative study was approved by the Hospital Infantil Albert Sabin Ethics Committee, accredited by CONEP - CNS/MS, Protocol No.67/2006 and conducted in accordance with the Brazilian Federal Law No. 11794 of October 8, 2008 (http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2008/Lei/L11794.htm) and Decree No. 6689 of July 15, 2009 , available at http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2009/Decreto/D6899.htm.

Twenty children with acyanotic congenital heart disease, scheduled for elective cardiac surgery with cardiopulmonary bypass, age range 1 day to 14 years were randomly assigned to 2 groups: Group GP, programmed to receive total intravenous anesthesia with propofol and group GS scheduled to use balanced anesthesia with sevoflurane. Exclusion criteria were cyanotic heart disease or complex, association with other malformations, severe systemic diseases, infection or children undergoing treatment and palliative or emergency surgery. Blood samples were collected at three different time-points: T0, after radial artery cannulation; T1, 30 minutes after cardiopulmonary bypass (CPB) launch and T2, at the end of surgical procedure. Parameters analyzed included thiobarbituric acid-reactive substance (TBARS), glutathione (GLN), lactate and pyruvate plasmatic concentrations

Anesthetic technique

All patients received midazolam 0.5 mg / kg orally, 30 to 45 minutes before the surgical procedure as premedication. Anesthesia was induced with midazolam maleate 0.3 mg / kg, sufentanil citrate 1 µg / kg and vecuronium bromide 0.15 mg/kg. After tracheal intubation, anesthesia maintenance was performed in group GP with a continuous infusion of propofol 250 µg / kg / min until the onset of cardiopulmonary bypass followed by 200 µg propofol / kg / min during the course of CPB and propofol 150 µg / kg / min after the return of cardiocirculatory function. Sufentanil 0.25 µg / kg / hour and intermittent doses of vecuronium were administered in association with propofol to maintain adequate muscle relaxation. In the GS group, anesthesia was maintained with sevoflurane 1-4%, associated with the same dose of fentanyl by continuous infusion and subsequent doses of vecuronium. A capillary membrane oxygenator was used. Blood and crystalloid perfusate, were programmed to maintain hematocrit between 29 and 30%, keeping constant the electrolyte concentrations of the cardioplegic solution used to make the cardiac arrest in asystole.

Biochemical analysis

Metabolites (Lactate, pyruvate)[16], GSH[17] and TBARS[18] were measured according to biochemical methods published elsewhere.

Statistical analysis

The results of this study were expressed as mean ± SD (standard deviation), followed by the number of observations (n). Student´s t test was used to analyze anthropometric data. Intra-group comparisons were made using Friedman´s test. The comparison of means between groups was performed using the Wilcoxon test.

Results

The homogeneity of the groups (age, weight, sex) was confirmed as no differences (p>0.05) were found comparing groups GP and GS (Table 1). Figure 1 depicts distribution of patients´ congenital heart diseases types and percentiles.

TABLE 1 – Demographics – Patients included in the study. Values expressed as mean ± SD.

Demographics	*Group P*	*Group S*
Age (years)	5.0 ± 3.12	5.1 ± 3.47
Weight (kg)	17.3 ± 10.1	17.3 ± 9.49
Sex (M/F)	4/6	7/3

Student´s *t* test e (p>0.05)
M : male F : female

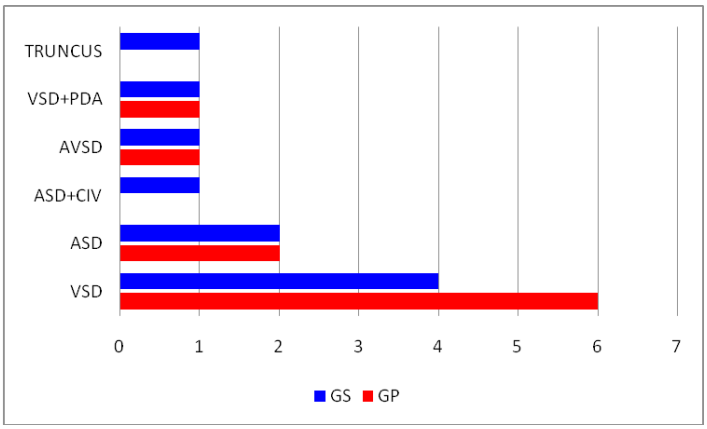

FIGURE 1 - Distribution of patients´ congenital heart diseases types and percentiles.

Ventricular septal defect (VSD): 50%
Atrial septal defect (ASD): 20%
Atrioventricular septal defect (AVSD): 10%
Truncus: 5%
Associations (ASD + VSD,PDA + VSD): 15%

MDA assay

MDA levels were not different in all timepoints within each group and between groups (Figure 2).

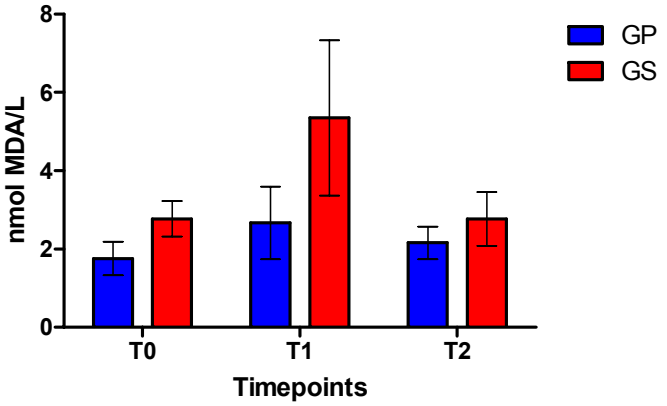

FIGURE 2 - Thiobarbituric acid-reactive substances levels (micromoles of Malondialdehyde per liter of fresh plasma) in children anesthetized with Propofol (GP) or Sevoflurane (GS). Bars represent mean±SD GP (blue bars) and GS (red bars) groups at T0, after radial artery cannulation, T1, 30 minutes after cardiopulmonary bypass launch and T2, at the end of the surgical procedure. Values in T0, T1 and T2 timepoints are not significantly different in each group by Friedman´s test. GS is not different from GP group by Wilcoxon test.

Reduced glutathione assay

GSH levels were not different in all timepoints within each group and between groups (Figure 3).

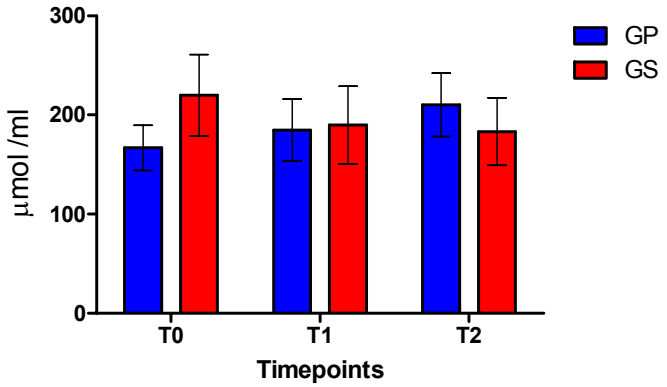

FIGURE 3 - GSH levels (micromoles per mililiter of fresh plasma) in children anesthetized with Propofol (GP) or Sevoflurane (GS). Bars represent mean±SD of GP (blue bars) and GS (red bars) groups at T0, after radial artery cannulation, T1, 30 minutes after cardiopulmonary bypass launch and T2, at the end of the surgical procedure. Values in T0, T1 and T2 timepoints are not significantly different in each group by Friedman´s test. GS is not different from GP group by Wilcoxon test.

Metabolites assay (Pyruvate and Lactate)

Pyruvate and lactate levels were not different in all timepoints within each group and between groups (Figures 4 and 5).

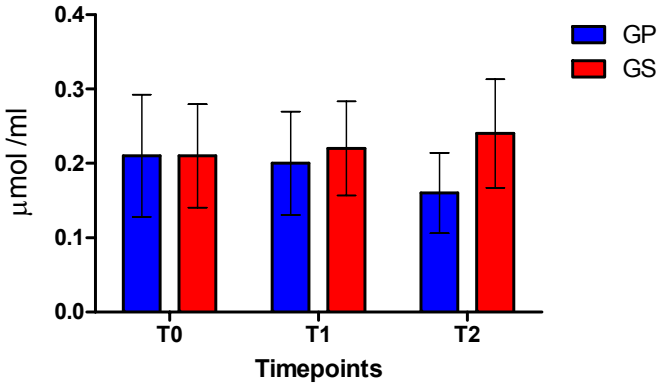

FIGURE 4 - Blood pyruvate levels (micromoles per mililiter) in children anesthetized with Propofol (GP) or Sevoflurane (GS). Bars represent mean±SD of GP (blue bars) and GS (red bars) groups at T0, after radial artery cannulation, T1, 30 minutes after cardiopulmonary bypass launch and T2, at the end of the surgical procedure. Values in T0, T1 and T2 timepoints are not significantly different in each group by Friedman´s test. GS is not different from GP group by Wilcoxon test.

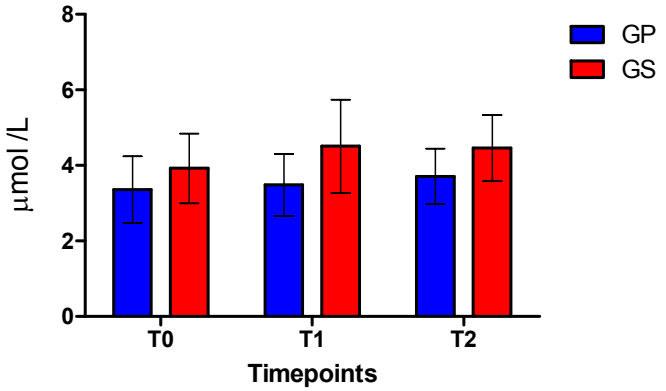

FIGURE 5 - Serum lactate levels (micromoles per liter) in children anesthetized with Propofol (GP) or Sevoflurane (GS). Bars represent mean±SD of GP (blue bars) and GS (red bars) groups at T0, after radial artery cannulation, T1, 30 minutes after cardiopulmonary bypass launch and T2, at the end of the surgical procedure. Values in T0, T1 and T2 timepoints are not significantly different in each group by Friedman´s test. GS is not different from GP group by Wilcoxon test.

Lactate / pyruvate ratio (L/P)

Lactate / pyruvate (L/P) means ratio was >10 in both groups. We found no significant difference comparing timepoints within groups (Friedman´s test). Also, ratio comparison between groups was not statistically significant (Table 2).

When performing correlation tests for the relationship between blood lactate and pyruvate (L / P ratio), a statistically significant high correlation was found, at the timepoint T1 (r = 0.83, p = 0.003). However, at the end of T2 the correlation was low (r = 0.28, p = 0.43) (Table 3).

TABLE 2 - Lactate / pyruvate (L/P) means ratio in children anesthetized with Propofol (GP) or Sevoflurane (GS). Values expressed as mean ± SD in both groups.

	Timepoint	*n*	*G P*	*G S*
L/P ratio	T0	10	34.29 ± 33.27	74.02 ± 147.97
	T1	10	31.31 ± 27.32	43,17 ± 73.91
	T2	10	59.22 ± 82.56	42.69 ± 43.49

Friedman and Wilcoxon tests (p>0.05)

TABLE 3 - Pearson correlation between groups GP and GS for lactate/pyruvate ratio (L/P).

	Correlation	*Timepoint*	*r*	*p*
L/P ratio	GP x GS	T1	0.83	0.003*
	GP x GS	T2	0.28	0.43

"r"=Pearson's Correlation Coefficient

Discussion

The comparison of two anesthetic techniques with different mechanisms of action, such as total intravenous anesthesia using propofol in continuous infusion, and the classic balanced anesthesia with sevoflurane, both associated with the same dose of opioid (sufentanil), provides the possibility of comparing the effects of these anesthetics drugs on the metabolic repercussions triggered by CPB for surgical correction of congenital heart disease.

There is a consensus in the literature that the intravenous anesthetic propofol and inhalational anesthetics such as sevoflurane have potent protective effects against the injury induced by ischemia-reperfusion[19]. However the two anesthetics have different mechanisms of action in the ischemic myocardium. The first reduces post-ischemic myocardial dysfunction, the ischemic area and histological degeneration. Furthermore, this intravenous anesthetic suppresses neutrophil activity, reduces free radicals and calcium influx[20], inhibiting the transitional mitochondrial permeability (a change in the pore of mitochondria), which can lead to apoptosis and necrosis[21]. Initially the proposed mechanisms for the cardioprotective effect of inhaled anesthetics were to preservation of ATP production, reduced calcium influx and inhibition of free radicals[19]. Recently, the elucidation of the action on coronary vasodilatation[22] by anesthetics such as sevoflurane, reinforces previous studies where it was demonstrated that the inhaled anesthetics act by preserving intracellular components such as potassium channel-dependent ATP (K_{ATP})[23], adenosine A1 receptors[24] and kinase C protein[25]. As a consequence of these intracellular events Sevoflurane improves post-ischemic contractile function, by mimicking pre-conditioning ischemia[26,27]. Malagon *et al*.[28] found no significant difference, in relation to heart surgery, in children undergoing cardiac surgery under sevoflurane and propofol anesthesias.

Currently, the arterial blood lactate is widely used as a marker of hemodynamic instability and in monitoring of tissue oxygen metabolism. During CPB, lactacemia tends to increase and may be influenced by changes in blood flow between the organs, the concentration of glucose, the use of catecholamines and Ringer solution[15]. In this study, there was no increase in the oxidative stress considering that MDA levels were not different comparing GP and GS groups. Although the plasma levels of TBARS in patients who received propofol as an anesthetic, was lower than the levels measured in the sevoflurane group, this difference was not significant. GSH levels were not different. Also, the blood concentration of lactate did not increase significantly throughout surgery, compared to baseline, observing during CPB (T1), an average blood concentration of less than 4 mmol / L (3.49 ± 2.60) in the GP group and greater than 4 mmol / L in GS (4.51 ± 3.90). We found a strong Pearson correlation between groups for lactate and pyruvate concentrations from lactate-pyruvate ratio (L / P), indicating that both groups had similar metabolic behavior.

Factors influencing the incidence, severity and recovery from oxidative and metabolic response that arises as a result of cardiac surgery and CPB, go beyond the effects of the anesthetic technique. The extent of surgical trauma, blood loss or transfusion, hypothermia and perioperative hemodynamic instability and bypass-related changes are involved in a positive outcome or not, to a lesser or greater degree[27].

Conclusion

Anesthetic techniques investigated in this study showed a similar pattern, with no increase in metabolic substrates and oxidative stress during surgical correction of congenital heart defects in non-cyanotic children.

References

1. Atik, FA. Monitorização hemodinâmica em cirurgia cardíaca pediátrica. Arq Bras Cardiol. 2004;82(2):199-208.
2. Azakie T, Merklinger SL, Mccrindle B, Van Arsdell GS, Lee KJ, Benson L N, Coles JG, Williams WG. Evolving strategies and improving outcomes of the modified norwood procedure: a 10-year single-institution experience. Ann Thorac Surg. 2001;72(4):1349-53.
3. Anand KJ, Hansen DD, Hickey PR. Hormonal-metabolic stress responses in neonates undergoing cardiac surgery. Anesthesiology. 1990;73(4):661-70.
4. Ballweg JA, Wernovsky G, Ittenbach RF, Bernbaum J, Gerdes M, Gallagher PR, Dominguez TE, Zackai E, Clancy RR, Nicolson SC, Spray TL, Gaynor JW. Hyperglycemia after infant cardiac surgery does not adversely impact neurodevelopmental outcome. Ann Thorac Surg. 2007;84(6):2052-8.
5. Falcao G, Ulate K, Kouzekanani K, Bielefeld MR, Morales JM, Rotta AT. Impact of postoperative hyperglycemia following surgical repair of congenital cardiac defects. Pediatr Cardiol. 2008;29(3):628-36.
6. Polito A, Thiagarajan RR, Laussen PC, Gauvreau K, Agus MS, Scheurer MA, Pigula FA, Costello JM. Association between intraoperative and early postoperative glucose levels and adverse outcomes after complex congenital heart surgery. Circulation. 2008;118(22):2235-42.
7. Jaggers J, Ungerleider RM. Cardiopulmonary bypass in infants and children. Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu. 2000;3:82-109.
8. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC. Isoflurane mimics ischemic preconditioning via activation of K(ATP) channels: reduction of myocardial infarct size with an acute memory phase. Anesthesiology. 1997;87(2):361-70.
9. Weber NC, Schlack W. Inhalational anaesthetics and cardioprotection. Handb Exp Pharmacol. 2008;(182):187-207.
10. Kevin LG, Novalija E, Stowe DF. Reactive oxygen species as mediators of cardiac injury and protection: the relevance to anesthesia practice. Anesth Analg. 2005;101(5):1275-87.
11. Xia Z, Huang Z, Ansley DM. Large-dose propofol during cardiopulmonary bypass decreases biochemical markers of myocardial injury in coronary surgery patients: a comparison with isoflurane. Anesth Analg. 2006;103(3):527-32.
12. Lunec J. Oxygen radicals: their measurement in vitro. Anal Proc.1989:26:130-1.
13. Cheung PY, Chui N, Joffe AR, Rebeyka IM, Robertson CM Postoperative lactate concentrations predict the outcome of infants aged 6 weeks or less after intracardiac surgery: a cohort follow-up to 18 months. J Thorac Cardiovasc Surg. 2005;130(3):837-43.
14. Duke T, Butt W, South M, Karl TR. Early markers of major adverse events in children after cardiac operations. J Thorac Cardiovasc Surg. 1997;114(6):1042-52.
15. Muñoz R, Laussen PC, Palácio G, Zienco L, Piercey G, Wessel DL. Changes in whole blood lactate levels during cardiopulmonary bypass for surgery for congenital cardiac disease: an early indicator of morbidity and mortality. J Thorac Cardiovasc Surg. 2000;119(1):155-62.
16. Hohorst HJD-Glucose-6-phosphate and Dfructose-6-phosphate. Determination withglucose-6-phosphate dehydrogenase and phosphoglucose isomerase. In: Bergmeyer HU. Methods of enzymatic analysis. London: Weinheim/Academic.; 1963. p.134-8.
17. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192-205.
18. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;86(1):271-8.

19. Ross S, Foëx P.Protective effects of anaesthetics in reversible and irreversible ischaemia-reperfusion injury.Br J Anaesth. 1999;82(4):622-32.
20. Sayin MM, Ozatamer O, Tasöz R, Kilinç K, Unal N. Propofol attenuates myocardial lipid peroxidation during coronary artery bypass grafting surgery. Br J Anaesth. 2002;89(2):242-6.
21. Sztark F, Ichas F, Ouhabi R, Dabadie P, Mazat JP. Effects of the anaesthetic propofol on the calcium-induced permeability transition of rat heart mitochondria: direct pore inhibition and shift of the gating potential. FEBS Lett. 1995;368(1):101-4.
22. Kawahito K, Kobayashi E, Ohmori M, Harada K, Kitoh Y, Fujimura A, Fuse K. Enhanced responsiveness of circulatory neutrophils after cardiopulmonary bypass: increased aggregability and superoxide producing capacity. Artif Organs. 2000;24(1):37-42.
23. Speechly-Dick ME, Grover GJ, Yellon DM. Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K+ channel? Studies of contractile function after simulated ischemia in an atrial in vitro model. Circ Res. 1995;77(5):1030-5.
24. Yao Z, Gross GJ. A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs. Efficacy, time course, and role of KATP channels. Circulation. 1994;89(3):1229-36.
25. Yang XM, Sato H, Downey JM, Cohen MV. Protection of ischemic preconditioning is dependent upon a critical timing sequence of protein kinase C activation. J Mol Cell Cardiol. 1997;29(3):991-9.
26. Novalija E, Fujita S, Kampine JP, Stowe DF. Sevoflurane mimics ischemic preconditioning effects on coronary flow and nitric oxide release in isolated hearts. Anesthesiology. 1999;91(3):701-12.
27. Kevin LG, Novalija E, Riess ML, Camara AK, Rhodes SS, Stowe DF. Sevoflurane exposure generates superoxide but leads to decreased superoxide during ischemia and reperfusion in isolated hearts. Anesth Analg. 2003;96(4):949-55.
28. Malagon I, Hogenbirk K, van Pelt J, Hazekamp MG, Bovill JG. Effect of three different anaesthetic agents on the postoperative production of cardiac troponin T in paediatric cardiac surgery. Br J Anaesth. 2005;94(6):805-9.

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