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Original Article

Effects of Remote Ischemic Preconditioning on Antioxidant Capacity and Lipid Peroxidation in Patients Undergoing Coronary Artery Bypass Grafting in Tehran Heart Center

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ABSTRACT

Background: Remote ischemic preconditioning (RIPC) may improve outcomes in ischemia/reperfusion injury (IRI) by improving antioxidant defense. We investigated total antioxidant capacity (TAC) and malondialdehyde (MDA) content as markers of lipid peroxidation.

Methods: The present randomized clinical trial allocated 50 coronary artery bypass graft (CABG) patients with cardiopulmonary bypass at Tehran Heart Center to 2 groups: RIPC and control (25 patients each). Clinical biochemistry parameters, TAC, and MDA were measured at 3 time points: post-anesthesia induction (before skin incision), immediately post-CPB, and 24 hours post-ICU admission.

Results: Increased transfusions of packed cells in the ICU and higher plasma MDA levels at post-CPB were observed in the control group. Additionally, significantly higher plasma TAC levels were observed at 24 hours post-ICU in the RIPC group.

Conclusions: RIPC protects against IRI in CABG on CPB by reducing lipid peroxidation and elevating antioxidant defense. RIPC could be integrated into CABG to reduce IRI adverse outcomes. ***(Iranian Heart Journal 2023; 24(1): 15-21)***

KEYWORDS: Coronary artery bypass, Ischemic preconditioning, Oxidative stress, Lipid peroxidation

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Cardiovascular diseases account for about 46% of all deaths in Iran and have been the leading cause of mortality among Iranians during the last 4 decades. [1] Coronary artery disease is the most prevalent cardiovascular disease in Iran, and coronary artery bypass graft surgery (CABG) for correcting the condition is the most

common operation. [1] Cardiopulmonary bypass (CPB) is a significant procedure in CABG, necessitating the use of cardioplegia for cardiac protection during the operation. [2] The cessation of heartbeat and myocardial perfusion happens with cardioplegia and results in an episode of ischemia. [3,4] Reperfusion injury happens following myocardial perfusion reestablishment and has local and systemic adverse effects, [5,6] such as enhanced anaerobic metabolism, oxidative stress, and systemic inflammatory response. [7,8] Ischemia-reperfusion injury (IRI) during CABG is a well-known factor in causing oxidative stress. [2] Various approaches, such as hypothermia, premeditation with various anesthetics and anti-inflammatory medications, and off-pump CABG, are applied to ameliorate the impact of IRI.[2,9-11] Despite the successful use of off-pump CABG in many cases, its popularization is limited by the paucity of competent surgeons and the devices required for the procedure, as well as the need for better revascularization with on-pump operations. [12] Therefore, CPB is currently the most popular approach amongst surgeons for CABG. [12] Remote ischemic preconditioning (RIPC) has attracted surgeons as a method for reducing CPB adverse outcomes in CABG. [6] The induction of RIPC is defined as short-term, nonfatal ischemia induction in the organ of interest before the main ischemia episode in order to enhance the body defense mechanisms to cope with major ischemia. During IPC, factors are generated due to the first-induced ischemic insults. The factors are released into the systemic circulation and induce preconditioning in the organs distant from the IPC site. This phenomenon, termed "RIPC", has prompted surgeons to use it in cardiac surgery settings. The most popular RIPC technique uses controlled ischemia induction in either the upper or lower limbs. [2] It is known that RIPC improves inflammatory response following CABG with CPB. [6] Since oxidative stress and lipid peroxidation contribute to post-CPB adverse outcomes, [13] we sought to investigate the effects of RIPC on total antioxidant capacity (TAC) and lipid peroxidation in patients undergoing CABG on CPB.

METHODS

Study Design

The current randomized controlled double-blinded clinical trial recruited patients undergoing elective CABG on CPB in Tehran Heart Center (Tehran, Iran). The convenient sampling method was applied. Fifty patients meeting the inclusion criteria were recruited. Then, the patients were randomly allocated to 2 groups: RIPC and 2) control. The study protocol was in compliance with the ethical codes of the Helsinki Declaration on medical studies including human subjects and was approved by the Ethics Committee of Tehran University of Medical Sciences (the Ethics Committee reference number: IR.TUMS.MEDICINE.REC.1399.270). The inclusion criteria consisted of signing written informed consent, being a candidate for elective isolated CABG, having normal upper limb examinations, and not having undergone surgical operations in the preceding 3 months or acute medical conditions during the preceding 3 months. Patients with known inflammatory disorders, diabetes, liver disease, kidney disease, or vitamin C and/or E and/or other antioxidant consumption were excluded from the study.

The study was registered at the Iranian Registry of Clinical Trials (IRCT) (the registration number: IRCT20201014049022N1).

Surgical Procedure

The anesthesia induction and maintenance protocol and the surgical procedures are described elsewhere. [14] In brief, pulse

oximetry and electrocardiography leads were monitored throughout the operation. Arterial blood pressure monitoring was conducted through a 20-gauge catheter. A double-cavity catheter was inserted into the right internal jugular vein prior to anesthesia induction to measure central venous pressure. Next, 5 µg/kg/h of fentanyl and 1 µg/kg/min of midazolam were administered for anesthesia maintenance during the operation after tracheal intubation. Heparin (300 IU/kg) was administered before aortic cannulation, and the activated clotting time was maintained above 480 seconds. CPB was established in a standard manner with the use of a roller pump and non-pulsatile flow (about 2.4 L/m^2 BSA/min according to blood pressure and body temperature). During CPB, the hematocrit level was maintained between 25% and 30%. Hypothermia (body temperature maintenance at 30 °C–32 °C) was applied. Hypotension correction was performed with phenylephrine infusion when MAP or systolic pressure reached 40 and 80 mm Hg, respectively.

A median sternotomy approach was used for surgery. The left internal mammary artery (with the pedicle) and the greater saphenous vein were harvested. The Ringer solution (10 mL/kg) was administered before surgery.

RIPC Induction

RIPC was induced as previously described. [6] Briefly, in the intervention group (RIPC), ischemia was induced by inflating the blood pressure cuff for 5 minutes; then, perfusion was reestablished by deflating the cuff for 5 minutes until the next round of ischemia. These cycles of ischemia and reperfusion in the upper limb were repeated 4 times.

Blood Sampling and Laboratory Analysis

Blood samples for measuring TAC and malondialdehyde (MDA) were drawn after the induction of anesthesia (before skin incision), following CPB (after weaning from CPB), and 24 hours after surgery (ICU admission). Plasma was isolated from heparinized blood samples with centrifugation at 3000 rpm for 10 minutes. Afterward, plasma was isolated and snap-frozen using liquid nitrogen and stored at −80 °C until the measurement of the desired parameters. TAC was measured using the Naxifer (Navand Salamat, Urmia, Iran) assay kit based on the ferric-reducing ability of plasma (FRAP) method. MDA measurement was performed using the Nalondi assay kit (Navand Salamat, Urmia, Iran) based on the thiobarbituric acid reactive substance (TBARS) method. All the measurements were in keeping with the suppliers' instructions. For the measurement of other factors, blood samples were directly transferred to the clinical laboratory at the study center and were analyzed using Hitachi 917 and 912 RA 1000 (Hitachi, Tokyo, Japan) devices with clinical biochemistry reagents (Pars Azmoon, Tehran, Iran).

Statistical Analysis

Data analysis was performed using the SPSS software version 18 (IBM Inc, CA, US). Continuous data were presented as the mean ± the standard deviation (SD) for data with normal distributions or medians (the interquartile range [IQR]) for data with non-normal distributions. Frequencies were compared using the χ^2 test. Continuous data with normal distributions were compared using the *t* test. Ordinal data or continuous data with non-normal distributions were compared using the Mann–Whitney *U* test. ANOVA with repeated measures was used to compare plasma TAC and MDA values, as well as creatinine and blood urea nitrogen, between and within the groups. Correlations were investigated using the Pearson or Spearman test according to the data distribution. The level of significance was 0.05, and the power of analysis was 0.8.

RESULTS

Fifty patients were enrolled for the current study and were randomly allocated into 2 groups of 25. The enrolled patients were diagnosed with coronary artery disease and indicated for CABG revascularization on CPB. The patients' demographics and descriptive data are presented in Table 1. No differences were observed between the groups in terms of perioperative outcomes, including the pump time, the CPB time, and the cross-clamp time. Additionally, the ICU length of stay and the mechanical ventilation time in the ICU were not statistically significantly different between the groups (Table 1). The use of packed red cells in the control group was higher in the ICU (P=0.022) (Table 2). No significant differences in blood chemistry parameters were observed (Table 2). The use of inotropic agents did not differ significantly between the 2 groups (Table 3). The measurement of the circulatory TAC level throughout the study demonstrated elevations in TAC in the RIPC group compared with the control group 24 hours after ICU admission (Table 4). The circulatory MDA level was significantly lower in the RIPC group than in the control group following CPB and 24 hours after ICU admission (Table 4). Cardiac troponin I (cTnI) values were similar in both groups.

Table 1: Study population's demographic characteristics and perioperative measures

Variables	RIPC	Control	*P* value
Age, y	61.24±7.49	64.41±9.8	0.242
Sex (male), %	72.7%	66.7%	0.747
Height, cm	167 (160-171)	168 (160-174.5)	0.375
Weight, kg	76 (66-83)	75.5 (71.5-84)	0.361
CPB time, min	67.86±23.08	74.18±30.83	0.452
Cross-clamp time, min	38.1±15.94	43.0±18.84	0.363
Mechanical ventilation, h	12 (10.5-12)	12 (9-12)	0.942
ICU stay, d	2.5 (2.25-3)	3.25 (2.5-5)	0.065

Table 2: Comparison of the study variables between the RIPC and control groups

Variables	Measurement Time	RIPC	Control	*P* value
BUN (mg/dL)	Preoperative	17 (14.5-20)	17 (12-31)	0.715
	Postoperative	17.5 (14.5-22)	16 (14-23)	0.763
	24 h postoperative	19.5 (16-24)	19 (14-31)	0.705
Creatinine (mg/dL)	Preoperative	1.0 (0.9-1.1)	1.0 (0.9-1.2)	0.939
	Postoperative	1.0 (0.8-1.1)	0.8 (0.6-1.2)	0.453
	24 h postoperative	1.0 (0.9-1.15)	1.0 (0.8-1.6)	0.790
CPK (U/L)	Preoperative	49.5 (31-118.5)	137.5 (86-179)	0.283
	Postoperative	266.5 (209.5-340)	321.5 (216-390)	0.549
	24 h postoperative	666 (511.5-876)	685.5 (428-827)	0.62
Packed cells (U)	OR	0.0 (0.0-2.0)	1.0 (0.0-1.0)	0.748
	ICU	0.0 (0.0-2.0)	2.0 (0.0-3.0)	0.022
Serum level of lactate	Preoperative	0.67±0.23	0.68±0.28	0.925
	After RIPC	1.1±0.82	1.14±0.84	0.884
	After CPB	2.01±1.09	1.91±1.08	0.755
	ICU admission	2.61±2.21	2.14±1.31	0.414
	6 hr post-ICU	2.63±2.72	2.88±2.34	0.753
Ejection fraction	Preoperative	40 (37.5-52.5)	50 (37.5-52.5)	0.188
	Postoperative	40 (32.5-47.5)	45 (35-50)	0.68

BUN, Blood urea nitrogen; CPK, Creatine phosphokinase; RIPC, Remote ischemic preconditioning; OR, Operating room; ICU, Intensive care unit

Table 3: Frequency of inotropic drug usage between the RIPC and control groups

Variables	Measurement Time	Control (n:22)	RIPC (n:21)	*P* value
Epinephrine	ICU	5	5	1.0
	OR	4	5	1.0
Norepinephrine	ICU	2	2	1.0
	OR	0	1	1.0
Dobutamine	ICU	0	0	-
	OR	0	0	-
Dopamine	ICU	0	0	-
	OR	0	0	-
Milrinone	ICU	0	0	-
	OR	0	0	-

RIPC, Remote ischemic preconditioning; OR, Operating room; ICU, Intensive care unit

Table 4: Study populations' plasma levels of systemic inflammatory markers and cardiac troponin I

Variables	Measurement Time	RIPC	Control	*P* value
TAC (mmol Fe^{2+}/L)	Baseline (before intervention)	0.44±0.16	0.42±0.18	0.62
	After CPB	0.45±0.2	0.42±0.16	0.47
	24 hours postoperative	0.48 (0.47-0.54)	0.45 (0.37-0.49)	0.034
MDA (nmol/mg protein)	Baseline (before intervention)	1.90±1.25	1.63±1.33	0.503
	After CPB	1.84±1.07	2.80±1.45	0.018
	24 hours postoperative	1.57±0.81	2.27±0.85	0.008
CTnI (ng/mL)	Baseline (before intervention)	0.25±0.19	0.26±0.23	0.97
	After CPB	4.51±4.01	4.17±3.99	0.56
	24 hours postoperative	2.23 ±1.14	2.02±2.77	0.48

CPB, Cardiopulmonary bypass; cTnI, Cardiac troponin I; RIPC, Remote ischemic preconditioning

DISCUSSION

In the current study, we investigated the effects of RIPC on systemic oxidative stress and lipid peroxidation in patients undergoing CABG on CPB. According to the findings, increased transfusions of packed cells in the ICU and higher plasma MDA levels at post-CPB were observed in the control group. Significantly higher plasma TAC at 24 hours post-ICU in the RIPC group was also observed.

Patients undergoing CABG on CPB are exposed to potent oxidative stress, which impairs their TAC [13] and elevates systemic and local inflammatory responses. [3,6,7] We speculated that protection against post-CPB IRI could be possible by improving TAC. Since RIPC is considered an approach to improving the outcomes of CPB, it is reasonable to assume that it has antioxidative stress effects. Antioxidant supplements and vitamins such as vitamins C and E [15, 16] have antioxidant properties and protect against oxidative stress-related adverse outcomes. RIPC activates the endogenous antioxidant defense system. It also acts through the modulation of the inflammatory cytokine response and reduces the systemic level of proinflammatory cytokines following CPB in patients undergoing CABG. [6]

Red blood cells are very sensitive to oxidation and lose their integrity in the face of severe oxidative stress. Since these cells are destroyed under oxidative conditions, presumably reductions in red blood cells and packed red cell transfusions are related to reduced TAC and enhanced lipid peroxidation. These can lead to red blood cell damage by hemolysis and lead to their removal by circulation. [17] Therefore, RIPC

may confer protection against oxidative stress by improving TAC and preventing lipid peroxidation. [2] It also reduces the anti-inflammatory cytokine response, [6] preventing a series of major adverse post-CPB events. This has probable positive impacts on the management of inflammation and oxidative stress and the related clinical outcomes following CPB.

CONCLUSIONS

RIPC protects against IRI in CABG on CPB by diminishing lipid peroxidation and augmenting the antioxidant defense. RIPC could be integrated into CABG to reduce the IRI adverse outcomes.

Authors' Contributions

All authors have participated in the idea conception and the intellectual content of the manuscript. Mohamadjavad Mehrabanian, Mehdi Dehghani Firoozabadi, Farhad Gorjipour, Hasan Soltaninia and Behrang Nooralishahi contributed by study design and protocol preparation. Mohamadjavad Mehrabanian, Farhad Gorjipour, Hasan Soltaninia, Behrang Nooralishahi, Mehdi Rahab and Masood Mohseni collected data, samples and perform the interventions. Mehdi Dehghani Firoozabadi supervised the entire research work. Behrang Nooralishahi, Mehdi Rahab and Masood Mohseni helped in the data interpretation. Mohamadjavad Mehrabanian performed statistical analysis and data presentation. Farhad Gorjipour prepared the first draft of the manuscript.
All the authors have read and approved the final version of the manuscript.

REFERENCES

1. Sarrafzadegan N, Mohammmadifard N. Cardiovascular disease in Iran in the last 40 years: prevalence, mortality, morbidity, challenges and strategies for cardiovascular prevention. Archives of Iranian medicine. 2019; 22(4):204-10.

2. Wang H, Lyu Y, Liao Q, Jin L, Xu L, Hu Y, et al. Effects of Remote Ischemic Preconditioning in Patients Undergoing Off-Pump Coronary Artery Bypass Graft Surgery. Frontiers in Physiology. 2019; 10(495).

3. Gorjipour F, Dehaki MG, Totonchi Z, Hajimiresmaiel SJ, Azarfarin R, Pazoki-toroudi H, et al. Inflammatory cytokine response and cardiac troponin I changes in cardiopulmonary bypass using two cardioplegia solutions; del Nido and modified St. Thomas': a randomized controlled trial. Perfusion. 2017; 32(5):394-402.

4. Mehrabanian MJ, Dehghani Firoozabadi M, Ahmadi Tafti SH, Forouzan Nia SK, Najafi A, Mortazian M, et al. Clinical Outcomes and Electrolyte Balance Factors in Complex Cardiac Operations in Adults; Del Nido® Versus Custodiol® Cardioplegia Solutions: A Randomized Controlled Clinical Trial. Iranian Red Crescent medical journal. 2018; 20(4):e64648.

5. Gorjipour F, Hosseini-Gohari L, Alizadeh Ghavidel A, Hajimiresmaiel SJ, Naderi N, Darbandi Azar A, et al. Mesenchymal stem cells from human amniotic membrane differentiate into cardiomyocytes and endothelial-like cells without improving cardiac function after surgical administration in rat model of chronic heart failure. Journal of cardiovascular and thoracic research. 2019; 11(1):35-42.

6. Gorjipour F, Saeedzadeh T, Toloueitabar Y, Kachoueian N, Bahlouli Ghashghaei S, Mortazian M, et al. Remote ischemic preconditioning effects on inflammatory markers and myocardial protection in coronary artery bypass graft surgery. Perfusion. 2020:0267659120979293.

7. Gorjipour F, Totonchi Z, Gholampour Dehaki M, Hosseini S, Tirgarfakheri K, Mehrabanian M, et al. Serum levels of interleukin-6, interleukin-8, interleukin-10, and tumor necrosis factor-α, renal function biochemical parameters and clinical outcomes in pediatric cardiopulmonary bypass surgery. Perfusion. 2019; 34(8):651-9.

8. Sadeghpour Tabaei A, Mortazian M, Yaghoubi A, Gorjipour F, Arasteh Manesh S, Totonchi Z, et al. Modified Ultrafiltration

in Coronary Artery Bypass Grafting: A Randomized, Double-Blinded, Controlled Clinical Trial. Iranian Red Crescent medical journal. 2018; 20(5):e66187.

9. Amani H, Mostafavi E, Alebouyeh MR, Arzaghi H, Akbarzadeh A, Pazoki-Toroudi H, et al. Would Colloidal Gold Nanocarriers Present An Effective Diagnosis Or Treatment For Ischemic Stroke? International journal of nanomedicine. 2019; 14:8013-31.

10. Pazoki-Toroudi HR, Hesami A, Vahidi S, Sahebjam F, Seifi B, Djahanguiri B. The preventive effect of captopril or enalapril on reperfusion injury of the kidney of rats is independent of angiotensin II AT1 receptors. Fundamental & clinical pharmacology. 2003; 17(5):595-8.

11. Zarch AV, Toroudi HP, Soleimani M, Bakhtiarian A, Katebi M, Djahanguiri B. Neuroprotective effects of diazoxide and its antagonism by glibenclamide in pyramidal neurons of rat hippocampus subjected to ischemia-reperfusion-induced injury. The International journal of neuroscience. 2009; 119(9):1346-61.

12. Khan MS, Islam MY, Ahmed MU, Bawany FI, Khan A, Arshad MH. On pump coronary artery bypass graft surgery versus off pump coronary artery bypass graft surgery: a review. Global journal of health science. 2014; 6(3):186-93.

13. Kunt AS, Selek S, Celik H, Demir D, Erel O, Andac MH. Decrease of total antioxidant capacity during coronary artery bypass surgery. The Mount Sinai journal of medicine, New York. 2006; 73(5):777-83.

14. Farsad BF, Janipour M, Totonchi Z, Gorjipour F, Omid SO. Effects of dexmedetomidine on surgical stress responses at patients under CABG. Biosciences Biotechnology Research Asia. 2016; 13(3):1537-45.

15. Rodrigo R, Korantzopoulos P, Cereceda M, Asenjo R, Zamorano J, Villalabeitia E, et al. A randomized controlled trial to prevent post-operative atrial fibrillation by antioxidant reinforcement. Journal of the American College of Cardiology. 2013; 62(16):1457-65.

16. Rodrigo R, Prieto JC, Castillo R. Cardioprotection against ischaemia/reperfusion by vitamins C and E plus n-3 fatty acids: molecular mechanisms and potential clinical applications. Clinical science (London, England : 1979). 2013; 124(1):1-15.

17. Maurya PK, Kumar P, Chandra P. Biomarkers of oxidative stress in erythrocytes as a function of human age. World journal of methodology. 2015; 5(4):216-22.