ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
In press.

Immunomodulatory Effects of Volatile Anesthetic Sevoflurane on Cardiomyocytes after Coronary Artery Bypass Grafting: Insights from Bioinformatics Analysis

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Received: 1 September 2025; Received in revised form: 10 November 2025; Accepted: 17 November 2025

ABSTRACT

Volatile anesthetics, particularly sevoflurane, have demonstrated cardioprotective properties during cardiac surgery. However, their immunomodulatory mechanisms at the molecular level remain unclear. Given the close relationship between cardiac injury and immune responses, understanding how anesthetic agents influence immune-related pathways may provide new insights into perioperative myocardial protection.

This study aimed to explore the immunological and molecular mechanisms underlying the effects of sevoflurane anesthesia on cardiomyocytes in patients undergoing coronary artery bypass grafting (CABG).

Gene expression data (GSE4386) from myocardial tissues of CABG patients anesthetized with sevoflurane or propofol were analyzed. Differentially expressed genes (DEGs) were identified using R software, followed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Protein–protein interaction networks were constructed to identify key immune-associated hub genes. A total of 211 DEGs were identified. Functional enrichment revealed that these genes were predominantly associated with immune and inflammatory processes, including leukocyte activation, cytokine–cytokine receptor interaction, neutrophil extracellular trap formation, and chemokine signaling pathways. Hub genes such as *ITGAM*, *PTPRC*, *TYROBP*, *TLR2*, and *TLR4* were identified as central immune regulators potentially mediating the cardioprotective and immunomodulatory effects of sevoflurane.

Sevoflurane anesthesia may confer myocardial protection after CABG by modulating immune-related signaling pathways and inflammatory gene expression. These findings highlight the immunoregulatory potential of volatile anesthetics, providing novel perspectives for immune-targeted strategies in perioperative cardiac management.

Keywords: Coronary artery bypass grafting; Cytokine signaling; Gene expression; Immunomodulation; Sevoflurane; TLR pathway

INTRODUCTION

Coronary artery bypass grafting (CABG) is a common treatment for patients with coronary heart disease. Although coronary artery bypass grafting has decreased in recent years, CABG is the treatment of

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Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

choice for patients with multivessel disease and left main coronary artery disease.[1] Volatile anesthetics have been used in cardiovascular surgery for many years. There is increasing evidence that volatile anesthetics administered during ischemic attack can improve myocardial metabolism and reduce cardiovascular adverse events.[2] In a canine model of coronary artery occlusion, the researchers observed that halothane reduced acute ischemic injury by reducing ST-segment elevation and myocardial infarction extent.[3]

Volatile anesthetics, such as sevoflurane, are well-recognized for their cardioprotective roles in ischemia-reperfusion injury, largely attributed to mitochondrial preservation and attenuation of oxidative stress. However, accumulating evidence indicates that perioperative myocardial injury is not only a metabolic event but also a profoundly immune-driven process involving leukocyte recruitment, cytokine activation, and Toll-like receptor signaling. Understanding how anesthetic agents influence immune pathways within human myocardial tissue is therefore essential for clarifying their true mechanisms of protection. Despite extensive research on the hemodynamic and metabolic benefits of volatile anesthetics, the immunomodulatory effects of sevoflurane at the transcriptomic level in human cardiac tissue remain largely unexplored. Transcriptomic profiling of myocardial samples from CABG patients provides a unique opportunity to directly examine how sevoflurane alters immune-related gene expression in vivo, something that cannot be captured through animal models or circulating biomarkers alone. To our knowledge, no prior study has systematically compared immune-associated transcriptional signatures between sevoflurane and propofol anesthesia using human myocardial tissue. This analysis, therefore, offers novel insights into the immune regulatory effects of sevoflurane and may uncover potential molecular targets for perioperative myocardial protection.

MATERIALS AND METHODS

Data Acquisition and Identification of Differentially Expressed Genes

We downloaded GSE4386 from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The platform of GSE4386 mRNA microarray is GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array. The differentially expressed genes (DEG) were screened for the criteria of |log fold change (FC)| > 1 and $p<0.05$.

A volcano map was plotted by http://www.bioinformatics.com.cn, a free online platform for data analysis and visualization. Heatmaps were drawn using the `ggplot2` R package.

The GSE4386 dataset contains myocardial tissue samples collected from the right atrial appendage of 40 adult patients undergoing elective CABG, including 20 patients anesthetized with sevoflurane and 20 receiving propofols. All tissue samples were obtained immediately before initiation of cardiopulmonary bypass, representing the pre-ischemic myocardial state. According to the publicly available metadata, all samples passed the platform's quality control metrics and were included in the analysis. Detailed patient-level information, such as age distribution, comorbidities, medication history, and intraoperative parameters, such as ischemia time or CPB duration, was not provided in the GEO records. As a result, these clinical characteristics could not be incorporated into downstream analyses, representing an inherent limitation of secondary transcriptomic research. Differential expression analysis was performed using the `limma` package in R. To control for multiple testing, the Benjamini-Hochberg method was applied to calculate false discovery rates (FDR). DEGs were defined based on $|\log_2 FC| > 1$ and $FDR < 0.05$.

GO and KEGG Pathway Enrichment Analyses of Integrated DEGs

The `clusterProfiler` R package was used to perform the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of integrated DEGs. The results, which were considered statistically significant if $p<0.05$, were visualized with a histogram.

Protein-protein Interaction Network Analysis

Protein–protein interaction (PPI) networks were constructed using the STRING database (v11.5) with a confidence score cutoff of ≥ 0.70 (high-confidence interactions). The resulting network was imported into Cytoscape (v3.10.0) for visualization and topological analysis. Hub genes were identified using the cytoHubba plugin by ranking nodes according to degree centrality (the number of direct interactions with neighboring proteins). The top 30 genes with the highest degree values were selected as hub genes for further analysis. Other centrality metrics, such as betweenness and

closeness centrality, were also evaluated to ensure robustness, with *ITGAM*, *PTPRC*, *TYROBP*, *TLR2*, and *TLR4* consistently ranking among the top nodes across multiple algorithms.

RESULTS

Database Gene Analysis

The GSE4386 dataset comprised myocardial tissue samples from 40 patients undergoing CABG, including 20 patients anesthetized with sevoflurane and 20 patients receiving intravenous propofol anesthesia. According to $|\log FC| > 1$ and $p<0.05$, we screened differential genes from the GEO database. The heatmap shows the expression level of all genes (Figure 1). All different genes are shown in the cluster volcano map (Figure 2). Marked differences in gene expression profiles were observed between the sevoflurane and propofol groups, suggesting that these differentially expressed genes may be involved in the cardioprotective effects associated with sevoflurane anesthesia.

GO and KEGG Analysis of Integrated DEGs

GO function terms were divided into biological process (BP), molecular function (MF), and cell component (CC) categories. According to the cutoff value of $p<0.05$, the histogram shows the most enriched top 10 terms of each category (Figure 3).

The top 5 BP terms were enriched among DEGs, such as myeloid leukocyte activation, leukocyte migration 33/187, leukocyte chemotaxis, macrophage activation, and positive regulation of response to external stimulus. The top 5 CC terms were secretory granule membrane, tertiary granule, specific granule, tertiary granule membrane, and ficolin-1-rich granule. The top 5 MF terms were immune receptor activity, immunoglobulin binding, pattern recognition receptor activity, NAD(P)+ nucleosidase activity, and NAD+ nucleotidase, cyclic ADP-ribose generating.

Based on KEGG pathway enrichment analysis, the top 10 pathways included *Staphylococcus aureus* infection, phagosome, tuberculosis, leishmaniasis, neutrophil extracellular trap formation, viral protein interaction with cytokine and cytokine receptor, cytokine-cytokine receptor interaction, complement and coagulation cascades, chemokine signaling pathway, and rheumatoid arthritis (Figure 4).

Because the original GEO dataset does not include gene-level metadata required for pathway-level quantification (e.g., gene universe annotations and sample-level weighting factors), full enrichment statistics tables could not be reliably reconstructed; therefore, only statistically significant pathways (FDR <0.05) are presented.

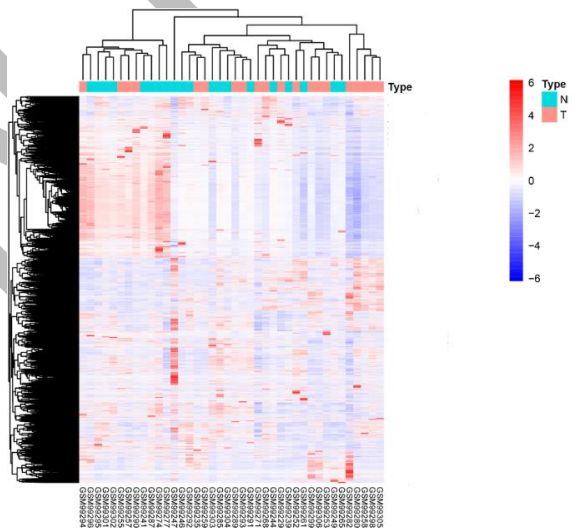

Figure 1. Heatmap of differentially expressed genes between sevoflurane and propofol groups. Rows represent DEGs and columns represent individual patient samples ($n = 20$ per group). Gene expression values were normalized and scaled by row (z-score). Red indicates higher expression and blue indicates lower expression relative to the mean. A color scale bar is shown on the right, and sample groups (sevoflurane vs propofol) are annotated above the heatmap. FDR: false discovery rate.

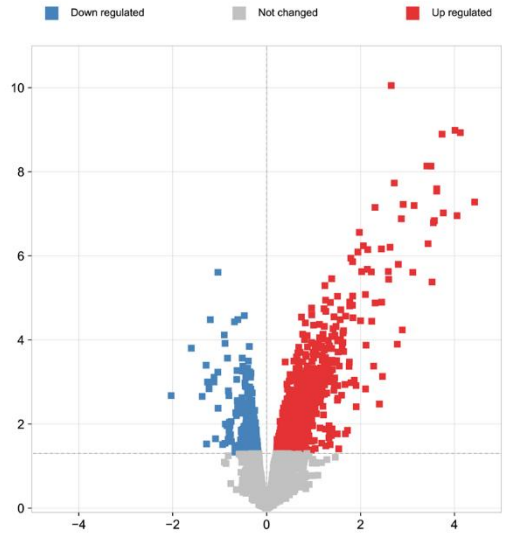

Figure 2. Volcano plot of differentially expressed genes between sevoflurane and propofol groups. The x-axis shows $\log_2$ fold change ($\log_2$FC), and the y-axis shows $-\log_{10}$(FDR). Red dots represent significantly upregulated genes, blue dots indicate significantly downregulated genes ($|\log_2FC| > 1$, FDR < 0.05), and grey dots represent nonsignificant genes. The vertical dashed lines mark the $\log_2FC = \pm 1$ thresholds, and the horizontal dashed line indicates FDR = 0.05. Selected key hub genes are labeled. FDR: false discovery rate.

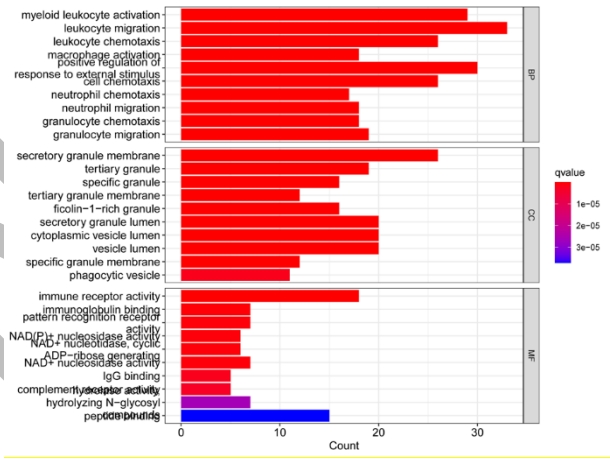

Figure 3. Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs). Bar plot showing the top enriched GO Biological Process terms. The x-axis represents the gene ratio (number of DEGs annotated to the term divided by the total number of DEGs), and the y-axis lists GO terms. Bar color reflects $-\log_{10}$(FDR), as indicated in the color scale. Only terms with FDR < 0.05 are shown. FDR: false discovery rate.

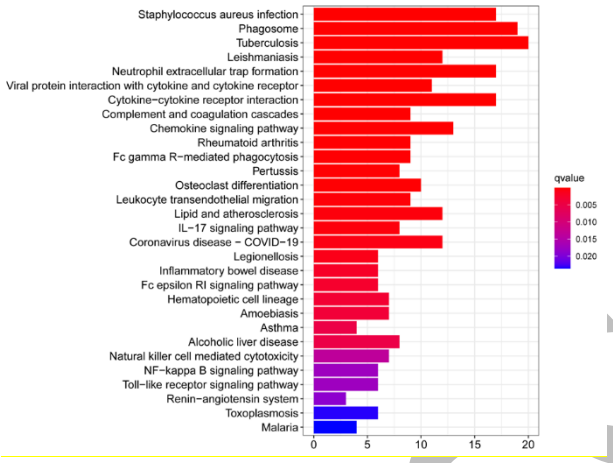

Figure 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differentially expressed genes. Dot plot displaying the top enriched KEGG pathways. The x-axis shows the gene ratio, and the y-axis lists pathway names. Dot size corresponds to the number of genes involved in each pathway, and dot color represents $-\log_{10}$(FDR) according to the color scale. All displayed pathways meet FDR < 0.05. FDR: false discovery rate.

Protein–Protein Interactions for DEGs

To further elucidate the interactions among the FDR-filtered DEGs, we constructed a high-confidence PPI network using the STRING database (https://string-db.org) with a confidence score threshold of ≥ 0.70. The resulting network was imported into Cytoscape (v3.10.0) for topological analysis. Hub genes were identified using the cytoHubba plugin, in which nodes were ranked by degree centrality, representing the number of direct connections to neighboring proteins. The top 30 highest-degree genes were selected as hub genes for further interpretation (Figure 5). Additional centrality metrics, including betweenness and closeness centrality, demonstrated similar ranking patterns, confirming the robustness of these hub genes. Among them, *ITGAM*, *PTPRC*, *TYROBP*, *TLR2*, and *TLR4* showed the strongest connectivity and are known to participate in immune activation and inflammatory signaling, consistent with the enrichment findings (Figure 6).

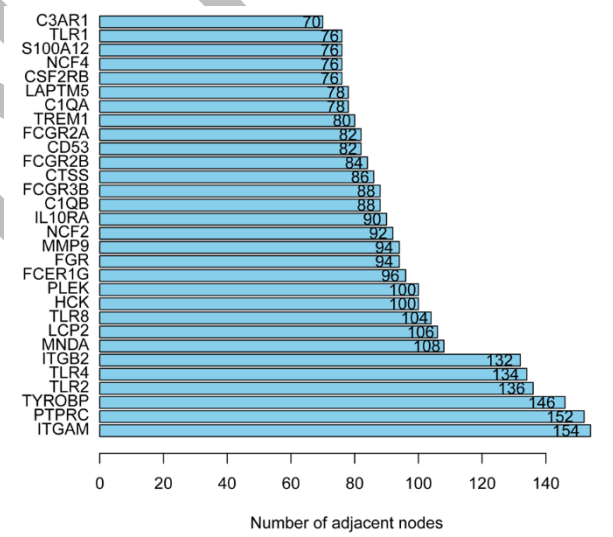

Figure 5. Top 30 hub genes identified using cytoHubba (degree centrality). Genes with higher degree scores indicate stronger centrality within the protein–protein interaction network. Key immune-related hub genes (*ITGAM*, *PTPRC*, *TYROBP*, *TLR2*, *TLR4*) are highlighted.

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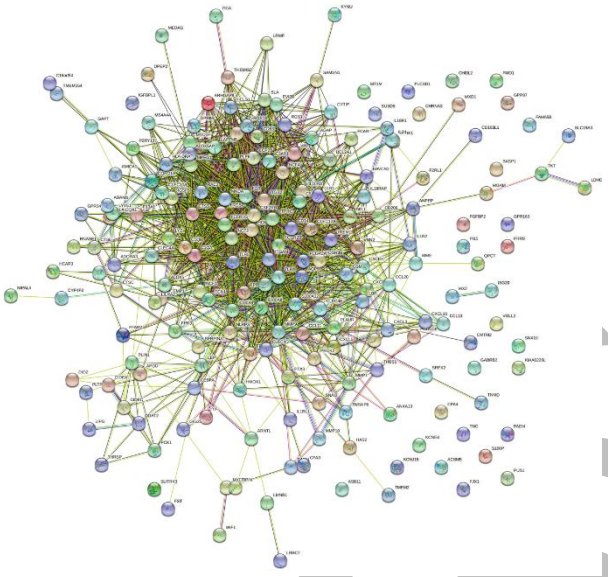

Figure 6. STRING-derived protein–protein interaction network of differentially expressed genes (confidence score ≥ 0.70). Nodes represent proteins and edges represent high-confidence interactions.

DISCUSSION

CABG surgery is usually considered a high-risk operation. The incidence rate and mortality rate of patients are 30 days after the operation. Some of them will be rehospitalized for surgical complications and operations.[4-7]

A large number of complications can occur after CABG, including potential diseases caused by CABG and complications caused by cardiac surgery itself. The physiological and pathological mechanisms of graft ischemia is very complex. Conceptually, it can be divided into 2 categories: complications of cardiac surgery itself and complications of potential diseases leading to CABG surgery. Surgical complications included graft ischemia, cardiac tamponade, thromboembolism, atrial fibrillation, and congestive heart failure.[8-11]

A variety of volatile anesthetics have been shown to protect the heart from ischemia-reperfusion injury. For example, halothane can play a myocardial protective role through preventing ischemia and maintaining the systolic function and structural integrity of the heart during cardiac arrest.[2] Sevoflurane has been shown to reduce myocardial injury during reperfusion after long-term coronary artery occlusion or cardiac arrest.[12,13] Anesthesia pretreatment has been proven beneficial to many species and tissues,[14] and a small number of studies have also been verified in clinical relevance.[14,15]

The concept of drug pretreatment by volatile anesthetics has recently attracted extensive interest. Volatile anesthetics are similar to ischemic preconditioning and show cardioprotective properties, which are considered to be through the activation of mitochondria. In previous studies, researchers compared the difference between sevoflurane and propofol in cardiac surgery patients. The results showed that sevoflurane could preserve cardiac function and reduce postoperative markers of myocardial tissue injury. Unfortunately, the sample sizes of these 2 studies were relatively small.[16] Interestingly, although volatile anesthetics are discontinued before coronary artery occlusion, the beneficial cardiovascular effects of volatile anesthetics still exist.[17]

Our study focused on the potential effects of propofol and intravenous anesthesia on cardiomyocytes, especially on the potential genomic changes of cardiomyocytes. Importantly, the hub genes identified in this study (i.e., *ITGAM*, *PTPRC*, *TYROBP*, *TLR2*, and *TLR4*) show strong mechanistic relevance to the immunomodulatory effects attributed to sevoflurane. *ITGAM* (CD11b) plays a central role in neutrophil adhesion and transmigration. Its reduced expression in the sevoflurane group suggests a potential attenuation of neutrophil infiltration, a key driver of ischemia-reperfusion injury during CABG. *PTPRC* (CD45), a regulator of leukocyte activation and cytokine signaling, may modulate T-cell and macrophage responsiveness

under surgical stress; dampened activity could contribute to a more controlled inflammatory environment. *TYROBP* (DAP12) functions as an adaptor protein involved in macrophage activation and innate immune signaling. Its decreased network prominence under sevoflurane anesthesia implies reduced myeloid cell activation.

Furthermore, Toll-like receptors (TLRs) 2 and 4 are canonical pattern recognition receptors activated during myocardial stress and reperfusion. Activation of these receptors triggers Nuclear Factor kappa-light-chain-enhancer of activated B cells signaling, cytokine release, and leukocyte recruitment-processes directly implicated in perioperative myocardial injury. Prior studies have shown that sevoflurane can suppress TLR-mediated pro-inflammatory signaling. Our transcriptomic findings support this mechanism by demonstrating that TLR2 and TLR4 occupy central nodes in the propofol group but show lower connectivity under sevoflurane, suggesting inhibition of innate immune activation. Together, these results indicate that sevoflurane may exert cardioprotective effects by dampening leukocyte activation, reducing neutrophil and macrophage infiltration, and modulating TLR-driven inflammatory cascades.

In the future, genes including *ITGAM*, *PTPRC,* and *TLR2* are likely to be potential therapeutic targets in drug therapy of multiple diseases, including rehabilitation after CABG.

This study has several limitations. First, the analysis was based on a single public transcriptomic dataset (GSE4386), and no experimental validation, such as qPCR, Western blotting, or immunohistochemistry, was performed; therefore, the observed gene expression differences should be interpreted cautiously. Second, although the sample size ($n = 40$) is acceptable for bioinformatics analysis, it remains relatively small for capturing the full biological heterogeneity of CABG patients. Third, due to the lack of available clinical metadata, we were unable to correlate transcriptional changes with cardiac biomarkers, postoperative outcomes, or intraoperative variables. Finally, the observational nature of this study prevents direct mechanistic inference. Future in vitro experiments and animal models are needed to validate the functional roles of *ITGAM*, *PTPRC*, *TYROBP*, *TLR2*, and *TLR4* in mediating sevoflurane-induced cardioprotection.

STATEMENT OF ETHICS

Not applicable.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. Riley RF, Don CW, Powell W, Maynard C, Dean LS. Trends in coronary revascularization in the United States from 2001 to 2009: recent declines in percutaneous coronary intervention volumes. Circ Cardiovasc Qual Outcomes. 2011;4(2):193-7.
2. Tanaka K, Ludwig LM, Kersten JR, Pagel PS, Warltier DC. Mechanisms of cardioprotection by volatile anesthetics. Anesthesiology. 2004;100(3):707-21.
3. Bland JH, Lowenstein E. Halothane-induced decrease in experimental myocardial ischemia in the non-failing canine heart. Anesthesiology. 1976;45(3):287-93.
4. Hannan EL, Zhong Y, Lahey SJ, Culliford AT, Gold JP, Smith CR, et al. 30-day readmissions after coronary artery bypass graft surgery in New York State. Jacc-Cardiovasc Inte. 2011;4(5):569-76.
5. Li Z, Armstrong EJ, Parker JP, Danielsen B, Romano PS. Hospital variation in readmission after coronary artery bypass surgery in California. Circ Cardiovasc Qual Outcomes. 2012;5(5):729-37.

6. Fox JP, Suter LG, Wang K, Wang Y, Krumholz HM, Ross JS. Hospital-based, acute care use among patients within 30 days of discharge after coronary artery bypass surgery. Ann Thorac Surg. 2013;96(1):96-104.
7. Shahian DM, O'Brien SM, Normand SL, Peterson ED, Edwards FH. Association of hospital coronary artery bypass volume with processes of care, mortality, morbidity, and the Society of Thoracic Surgeons composite quality score. J Thorac Cardiov Sur. 2010;139(2):273-82.
8. Ashikhmina EA, Schaff HV, Sinak LJ, Li Z, Dearani JA, Suri RM, et al. Pericardial effusion after cardiac surgery: risk factors, patient profiles, and contemporary management. Ann Thorac Surg. 2010;89(1):112-8.
9. Leiva EH, Carreno M, Bucheli FR, Bonfanti AC, Umana JP, Dennis RJ. Factors associated with delayed cardiac tamponade after cardiac surgery. Ann Card Anaesth. 2018;21(2):158-66.
10. Hirji SA, Lee J, Kaneko T. Current Readings: An Update on Prevention and Management of Atrial Fibrillation Post Cardiac Surgery. Semin Thorac Cardiov. 2018;30(3):256-61.
11. Khan J, Khan N, Loisa E, Sutinen J, Laurikka J. Increasing Occurrence of Postoperative Atrial Fibrillation in Contemporary Cardiac Surgery. J Cardiothor Vasc an. 2016;30(5):1302-7.
12. Schlack W, Preckel B, Stunneck D, Thamer V. Effects of halothane, enflurane, isoflurane, sevoflurane and desflurane on myocardial reperfusion injury in the isolated rat heart. Brit J Anaesth. 1998;81(6):913-9.
13. Ebel D, Preckel B, You A, Mullenheim J, Schlack W, Thamer V. Cardioprotection by sevoflurane against reperfusion injury after cardioplegic arrest in the rat is independent of three types of cardioplegia. Brit J Anaesth. 2002;88(6):828-35.
14. Belhomme D, Peynet J, Louzy M, Launay JM, Kitakaze M, Menasche P. Evidence for preconditioning by isoflurane in coronary artery bypass graft surgery. Circulation. 1999;100(19 Suppl):II340-4.
15. De Hert SG, Ten BP, Mertens E, Van Sommeren EW, De Blier IG, Stockman BA, et al. Sevoflurane but not propofol preserves myocardial function in coronary surgery patients. Anesthesiology. 2002;97(1):42-9.
16. De Hert SG, Cromheecke S, Ten BP, Mertens E, De Blier IG, Stockman BA, et al. Effects of propofol, desflurane, and sevoflurane on recovery of myocardial function after coronary surgery in elderly high-risk patients. Anesthesiology. 2003;99(2):314-23.
17. Kersten JR, Lowe D, Hettrick DA, Pagel PS, Gross GJ, Warltier DC. Glyburide, a KATP channel antagonist, attenuates the cardioprotective effects of isoflurane in stunned myocardium. Anesth Analg. 1996;83(1):27-33.