Original Article

Expression Profiles of P53, Caspase-3 And Bcl-2 in Patients Undergoing Congenital Heart Corrective surgery: Combined Effects Of Anesthesia And Surgery

Ahmed Farouk[1], Rasha Hamed[2], Mohamed H. Hassan[3], Farag Moftah[4], Tahia H. Saleem[5], Saeid Elsawy[6*]

Abstract

Background: Apoptosis is a physiological programmed cell death necessary for the development and cellular homeostasis. Dysregulation of apoptosis pathways leads to several diseases such as cancer, autoimmune and immunodeficiency diseases, and neurodegenerative disorders.

Materials and Methods: The current study included 56 patients (29 males and 27 females), undergoing corrective heart surgery operations, categorized into 3 groups: group A included 23 patients with atrial septal defect (ASD); group B included 15 patients with ventricular septal defect (VSD), and group C included 15 patients with Fallot tetralogy. Biochemical assays of apoptotic (P53 and caspase 3) and antiapoptotic markers (Bcl-2) using colorimetric and ELISA assay kits were performed on all included patients twice, preoperative and 24 hours postoperative.

Results: There was no statistically significant difference in the preoperative levels of Bcl-2, Caspase-3, and P53 between the three groups. While the significant difference was found when comparing the preoperative and postoperative levels of the previous markers in the same group and between groups ($p<0.05$ for all).

Conclusion: Both apoptotic and antiapoptotic pathways are activated during congenital heart corrective surgeries, increased markers directly related to the duration of anesthesia exposure.

Keywords: P53, Caspase-3, Bcl-2, Congenital Heart Surgery, Apoptosis

1. Cardiothoracic Surgery Department, Faculty of Medicine, Assiut University, Assiut, Egypt
2. Anesthesia and Intensive Care Department, Faculty of Medicine, Assiut University, Assiut, Egypt
3. Medical Biochemistry Department, Faculty of Medicine, South Valley University, Qena, Egypt
4. Community Medicine and Medical Statistics Department, Faculty of Medicine, Assiut University, Assiut, Egypt.
5. Medical Biochemistry Department, Faculty of Medicine, Assiut University, Assiut, Egypt
6. Anesthesia and Pain Management Department, Faculty of Medicine, Assiut University, Assiut, Egypt

Corresponding Author: S
Ahmed Farouk, M.D, Associate Professor, Department of Anesthesia, ICU and pain management, Faculty of Medicine, Assiut University, Assiut, Egypt.
E-mail address: ahmedf@aun.edu.eg

Please cite this article as: Farouk A, Hamed R, Hassan MH, Moftah F, Saleem TH, Elsawy S. Expression Profiles of P53, Caspase-3 And Bcl-2 in Patients Undergoing Congenital Heart Corrective surgery: Combined Effects Of anesthesia And Surgery. J Cell Mol Anesth. 2021;6(4):286-93. DOI: https://doi.org/10.22037/jcma.v6i4.36022

Introduction

Congenital heart condition is a serious problem facing pediatrics. Corrective surgery may be a challenge that needs a major surgical intervention with protracted anesthesia exposure. Apoptosis is defined as a programmed physiological cell death essential for cellular development and homeostasis (1). Disruptions of apoptosis pathways result in various diseases like cancer, autoimmune and immunodeficiency diseases, and neurodegenerative disorders (2). Apoptosis has shown to be involved in each acute and chronic cardiomyocyte loss in myocardial infarction, reperfusion injury, cardiomyopathies, and acute and chronic heart failure progression. The current debates area is what types of cell death (apoptotic vs. non-

apoptotic) predominate in chronic cardiac disease and whether or not the prevention of cell death by chronic inhibition therapy could be beneficial in deterring the advancement of heart failure. Apoptosis is a tightly regulated process that requires strict interactions between various pro-and anti-apoptotic molecules. Several different mechanisms have been described, such as intrinsic vs. extrinsic pathway or caspase-dependent vs. caspase-independent apoptosis. The caspases are a family of cysteine proteases that cleave specific proteins at specific aspartate residues. Initiator caspases (caspase 9 – 8) possess a long prodomain with a functionally important interacting domain; they act upstream to initiate and regulate apoptosis and downstream to activate effector caspases. Effector caspases, such as caspase-3, are characterized by short pro-domains and rely upon initiator caspases for activation. Principally, caspase-mediated apoptosis is mediated by extrinsic (involving death receptors) or intrinsic (mitochondria-mediated) pathways. These two pathways usually converge on a common effector caspase, such as caspase-3, to finalize the morphologic and biochemical alterations which characterize apoptosis. The binding of a death ligand initiates death receptor-mediated pathway, e.g., Fas ligand (FasL) or tumor necrosis factor-alpha (TNF-α) to a membrane-bound death receptor (e.g., Fas or TNF-α receptor). This interaction results in the recruitment of a death domain, e.g., Fas-associated death domain (FADD), which activates caspase-8 followed by the downstream effector caspases.[3] The intrinsic cell cycle pathway is initiated at the mitochondrial level and includes mainly P53, induced by broken DNA. The induction of P53 by damage initially activates DNA repair enzymes expression. If DNA repair is not effective, then other proteins are stimulated by P53 (P53 cofactors) will induce cell cycle arrest (programmed cell death).[4]Mitochondria is a vital organism that constitutes about 30% of cardiomyocytes volume and responsible for adenosine triphosphate (ATP) for cellular function. However, mitochondria become a vital organelle in the initiation of the cell cycle when apoptosis is triggered, as in oxidative stress, in the intrinsic mitochondrial apoptosis pathway. The apoptotic insult induces mitochondria to release cytochrome C into the cytoplasm. Their mitochondria form an activation complex named apoptosome. Apoptosome formation contributes to the auto-processing of caspase-9 and activation of downstream caspases such as caspase-3. The regulation of apoptotic factors released from mitochondria, such as cytochrome c, is balanced by the Bcl-2 protein family. The Bcl-2 family of proteins can be categorized as either anti-apoptotic (e.g., Bcl-2 and Bcl-xL) or pro-apoptotic (e.g., Bad, Bak, and Bax) (3). Cardiopulmonary bypass generates factors that induce apoptosis, such as oxygen free radicals, TNF-α, nitric oxide, neurohumoral factors as angiotensin II (4). Moreover, mechanical stress and hypothermia induce apoptosis (5). Conflicting results are present regarding the role of anesthetics in apoptosis induction. Sevoflurane, which is the mainstay of inhalational anesthesia nowadays, especially in pediatric populations. Some studies reported that sevoflurane leads to apoptosis in the developing brain and causes long-term cognitive deficits. However, a more recent study found that sevoflurane could promote hippocampal neurogenesis in neonatal rats and facilitate their performance in dentate gyrus-dependent learning tasks (6). Propofol, IV anesthetics with non-anesthetic actions, meliorates oxidative damage, reduces pro-inflammatory cytokines, inhibits cyclooxygenase -2 and prostaglandin E2 functions, and minimizes surgery-induced surgery stress responses, lowers immunosuppression after, propofol promote apoptosis and cell cycle arrest of many cancer cells (7). Some literature proposed that propofol could induce apoptosis (8-11). Few pieces of the literature suggested that propofol could inhibit apoptosis (11-13). The balance between pro-apoptotic and anti-apoptotic proteins is crucial for cell survival (14). Therefore, we aimed to investigate the effect of congenital heart corrective surgeries and associated anesthesia on the apoptotic pathway by measuring levels of both the apoptotic and antiapoptotic markers caspase-3, P53, and BCL-2, preoperative and postoperative.

Methods

Study design and participants: A clinical longitudinal study that was carried out in Assiut University, Faculty of Medicine, Cardiothoracic Surgery Department in collaboration with Medical Biochemistry Departments, Faculties of Medicine,

Assiut and South Valley Universities, Egypt. Institutional board review registration was obtained No. 13700420 and clinical trial registration NCT04431505. Patient consent to participate was obtained from their parents before the operation. The study included 56 patients divided into three groups; Group A: included 23 patients undergone ASD correction. 15 females and 8 males their ages ranged from 13 to 18 years old. Group B: included 18 patients undergone VSD correction.11 males and 7 females, their ages ranged from 3 to 9 years old. Group C: included 15 patients undergone fallout tetralogy (FT) correction. 10 males and 5 females, their ages ranged 3 to 7 years old.

Preoperative investigations

1. Full history and clinical examination.
2. Routine laboratory investigations (CBC, RFTs, LFTs, PT and prothrombin concentration, RBS and electrolytes)
3. ECG, X-Ray and Echocardiography, cardiac catheterization when indicated.

Anesthesia Technique: All patients received the same general anesthesia protocol, Induction done by propofol (1-2 mg/kg), titrated slowly intravenous till loss of verbal contact, cisatracurium 0.15mg/kg, fentanyl 2mic/kg, and lidocaine 1.5 mg/kg. The following induction; embedding arterial line, the central venous catheter (CVP), and urinary catheter was inserted. Maintenance of anesthesia is done by mechanical ventilation-sevoflurane anesthesia, fentanyl infusion, and muscle relaxant. Monitoring is done by IBP, ECG, pulse oximetry, UOP, and temperature. For patients on cardiopulmonary bypass, maintenance of anesthesia continues by propofol infusion and muscle relaxant.

Surgical approach: For congenital heart corrective surgeries, patients were approached via a median sternotomy approach. Standard aortic and bicaval cannulation was done. An initial dose of 400 μg/kg heparin was used to obtain an activated clotting time. Blood pressure range kept at 50-60 mmHg and blood flow 2-2.4 L/min. The priming solution contained mannitol and heparin. Hypothermia for 25-30 minutes. Cold crystalloid cardioplegic (4°C) arrest was used after aortic cross-clamping. ICU follow-up for hemodynamic changes in the heart rate, arterial blood pressure, and central venous pressure. Antipyretics and potent antibiotic therapy were given before discharge patient must meet the following: mindful, impulsive breathing, detached endotracheal tube.

Blood samples and biochemical assays: 5ml venous blood was withdrawn from each participant immediately preoperative and 24 hours postoperative. Samples were divided into 3ml in serum gel separator tube and 2ml in EDTA tube. The sera are then separated and kept at -80ºC till the time of Bcl-2 and P53 assay. For caspase 3 activities, RBCs isolated from EDTA tube after washing, hemolysate is obtained and deeply frozen until the time of estimation.

A. Bcl-2 and P53 were estimated by microplate ELISA reader (EMR-500, Labomed Inc., USA) using commercially available ELISA assay kits supplied by Endogene corroborating MA USA cat. NO.EH-Bcl-2 and Biosource international (Europe SA) catalog KH 00151 California USA, respectively. Normal reference ranges for Bcl-2 and P53 are (28.5- 36 ng/mL; 231-514 pg/mL respectively

B. Caspase-3 activity in lysates was performed using CAPO target in caspase -3 protease colorimetric assay kit (cat. No. KHZ0022 Biosource Europe A. J Belgium). The activity of caspase-3 was calculated as a ratio of OD of caspase-3 to protein concentration (in ug). Normal reference range for caspase-3 is (0.0021-0.0003 ng/mL).

Statistical analysis: All statistical analyses were performed using IBM SPSS statistics version 22 (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk test assessed the distribution of baseline variables. Paired sample and independent sample T-test were used for the analysis of parametric data. Probability values of $P \leq 0.05$ were considered significant.

Results

The high statistically significant difference was observed between the preoperative and postoperative levels of three markers in all operations; mean Bcl-2 levels in ASD, VSD, and FT was (26±6.89 *vs.* 53±8.86 $p<0.001$, 23.83±5.9 *vs.* 57.28±10.5 $p<0.001$ and 25.73± 6.6*vs.*97.20 ± 8.9 $p<0.001$ respectively) with the highest elevation observed in Fallout Tetralogy group. Also, Caspase-3 levels significantly increased postoperative compared to preoperative levels in ASD, VSD, and FT, with the highest elevation, also occurred (0.0033 *vs.* 0.0037 $p<0.001$, 0.0032 *vs.* 0.0038 and 0.0032 *vs.*0.0042 $p<0.001$ respectively). Furthermore,

Table 1: Comparison between preoperative and postoperative circulating levels of apoptosis biomarkers among study groups.

	Preoperative (mean±SD)	Postoperative (mean±SD)	P value
ASD (n=23)			
Bcl-2 (ng/mL)	26±6.89	53±8.86	<0.001**
Caspase (ng/mL)	0.0033±0.0001	0.0037±0.0001	<0.001**
P53 (pg/mL)	427±149	1219±169	<0.001**
VSD (n=18)			
Bcl-2(ng/mL)	23.83±5.9	57.28±10.5	<0.001**
Caspase-3 (ng/mL)	0.0032±0.00003	0.0038±0.00033	<0.001**
P53 (pg/mL)	434.2±144.6	1512.7±149.9	<0.001**
Tetraology of Fallot (n=15)			
Bcl-2 (ng/mL)	25.73± 6.6	97.20 ± 8.9	<0.001**
Caspase-3 (ng/mL)	0.0032± 0.003	0.0042±0.003	<0.001**
P53(pg/mL)	405.60±111	2009.53±118	<0.001**

Independent sample T test was used.

levels of P53 followed the same course with highly significant elevation in the postoperative levels than the preoperative levels in ASD, VSD, and FT groups (427±149 *vs.* 1219±169 p<0.001, 434.2±144.6 *vs.* 1512.7±149.9 and 405.60±111; p<0.001 *vs.* 2009.53±118; p<0.001, respectively) (**Table.1**).

For Bcl-2, no statistical significance was detected in the preoperative levels between the three groups (P1=0.3, P2=0.9, and p3=0.4). Also, no statistical difference was found between the postoperative levels of Bcl-2 between ASD and VSD P1=0.16. While, a highly significant difference was found in the postoperative levels of Bcl-2 between FT group and ASD and VSD P2=<0.001, P2<0.001, **(Table 2).**

Caspase-3 tends to follow the same course of Bcl-2, where no statistical significance was detected between the three groups (P1=0.5, P2=0.4, and p3=0.9). Also, no statistical difference was found between the postoperative levels of caspase-3 between ASD and VSD P1=0.46. While a high significant difference was found in the postoperative levels of Caspase-3 between the FT group and ASD and VSD P2=<0.001, P2<0.001 **(Table 2).**

For P53, no statistical significance was detected in the preoperative levels between the three groups (P1=0.9, P2=0.6, and p3=0.5). However, a high statistical difference was found between the postoperative levels of P53 between the three groups P1=<0.00, P2=<0.001, and P2=<0.001 **(Table 2)**.

Discussion

Table 2: Comparison between postoperative and postoperative biomarkers levels between the study groups.

Bcl-2 (Mean ±SD, ng/mL)						
	ASD (=23)	**VSD (18)**	**FT (15)**	**P1**	**P2**	**P3**
Preoperative	26±6.89	23.83±5.9	25.73± 6.6	**0.3**	**0.9**	**0.4**
Postoperative	53±8.86	57.28±10.5	97.20 ± 8.9	**0.16**	**<0.001****	**<0.001****
Caspase-3 (Mean ±SD, ng/mL)						
	ASD	**VSD**	**FT**	**P1**	**P2**	**P3**
Preoperative	0.0033±0.00	0.0032±0.00003	0.0032± 0.003	**0.5**	**0.4**	**0.9**
postoperative	0.0037±0.00	0.0038±0.00033	0.0042±0.003	**0.46**	**<0.001****	**0.001****
P53 (Mean ±SD, pg/mL)						
	ASD	**VSD**	**FT**	**P1**	**P2**	**P3**
Preoperative	427±149	434.2±144.6	405.60±111	**0.9**	**0.6**	**0.5**
Postoperative	1219±169	1512.7±149.9	2009.53±118	**<0.001****	**<0.001****	**<0.001****

P1 comparison between ASD and VSD; P2 comparison between ASD and FT ; P3 comparison between VSD and FT

Apoptosis is an organized and sometimes energy-dependent process that implicates activation of cysteine proteases, a complicated cascade of events that targets cellular homeostasis maintenance. Apoptosis is different from necrosis. Cell necrosis results from nonspecific insult or toxic conditions, while apoptosis is an active signal-dependent cellular process. LÉVY et al. reported that impaired pulmonary endothelial cell apoptosis is associated with intimal proliferation and irreversibility of pulmonary hypertension in congenital heart disease (15).

Cellular death by apoptosis shows characteristic morphologic changes in membrane ruffling cytoplasmic condensation, nuclear contraction, and DNA cleavage. The resulting cellular fragments are ingested by phagocytes (4, 16). It is known that caspase and Bcl-2 families are the leading families responsible for apoptosis. Caspase-3 is the pivot executioner to the 'extrinsic' and "intrinsic apoptosis pathways. Bcl-2 or Bax is the vital antiapoptotic or proapoptotic protein induced by stress stimulation (2). It converges the apoptosis-related signal pathways. Once activated, caspases usually induce other pro-caspases and promote protease cascade, thereby irreversibly intensifying the apoptotic pathway. At present, more than ten caspases have been reported and recognized as initiators (caspase-2, 8, 9, and 10), executioners (caspase-3, 6, and 7), or other regulators (caspase-11, 12, 13, and 14) in apoptosis (17).

The cellular polypeptide that is cleaved by proteases is responsible for most cellular, and morphological events that happened during cell death caspases also promote the propensity for apoptosis (18, 19). Tumor suppressor genes named retinoblastoma (RB) and P53 genes are the negative regulators, which arrest the cell cycle. P53 is a multi-functional protein stimulated by damaged DNA in cells. When DNA damage is recognized, p53 halts the cell cycle and recruit's specific enzymes to repair the damaged DNA. If DNA repair is not possible, p53 triggers apoptosis,

to prevent duplication of damaged chromosomes. P53 is implicated in the transcriptional activation of BCL-2 protein family products.[14] This family includes BAX and BCL-2, which are essential in apoptosis regulation. BAX is the Pro-apoptotic agent, while BCL-2 is the anti-apoptotic one; overexpression antagonizes the other's effect (20).

The present study showed a significant increase in the postoperative levels of the previously mentioned parameters, compared to the baseline levels, according to the operation time. Hence, levels were more elevated in Fallout Tetralogy patients compared to ASD and VSD (P1=0.16, P2<0.001, P3<0.001). On the other hand, there was no significant difference in the preoperative levels between the study groups. Bcl-2 and Caspase-3 reflect the extrinsic pathway of apoptosis as antiapoptotic and apoptotic markers. A specific ligand-receptor interaction can induce apoptosis as Fas receptors (TNF), cytokines, and free radicals (15, 21).

P53 levels, the marker of the intrinsic apoptotic pathway, showed a significant postoperative increase in the three study groups compared to the preoperative levels. Moreover, the fallout group showed more increase than ASD and VSD groups. Surgical trauma is accompanied by the induction of TNF, IL6, IL-IB, and many oxygen free radicals (22). Recently, it was reported that peripheral blood mononuclear cells from patients undergoing surgical trauma are susceptible to accelerated Fas-mediated apoptosis (22).

The present study revealed that congenital heart disease surgery (ASD, VSA, and FT) with different prolonged exposure to general anesthesia showed a battle between apoptotic and anti-apoptotic markers. The length of anesthesia exposure is thought to be the cornerstone in this game. In agreement with our result; Xing et. al. reported that propofol induces apoptosis of non-small cell lung cancer by, either, inhibiting phosphorylation of extracellular signal-regulated kinase1/2 (ERK)or activation of the p53-upregulated modulator of apoptosis (PUMA) signaling, leading to apoptosis (24). Berns et.al. reported that exposure of the primary immature neuronal cells to isoflurane, but not fentanyl, leads to reduced cell viability and induces apoptosis. Isoflurane-induced apoptosis, which affects the immature but not the mature neuronal cells, could be inhibited by the pan-caspase inhibitor. Also, they suggested that the GABA-A receptor pathway may be involved in this inhibition (25). Nouguchi et.al. found that isoflurane anesthesia for 3 hours or more triggers apoptotic cell death in the macaque brain. The authors added that the amount of apoptosis increases increasing the duration of exposure, suggesting that the severity of the toxic action of isoflurane is proportional to the duration of exposure (26). Xy et. al. evaluated the effect of thoracic epidural (TEA) and propofol versus sevoflurane anesthesia with opioid analgesia in patients undergoing colon cancer surgery. They reported that serum samples from patients who received TEA inhibited proliferation and invasion of lovo cells and induced apoptosis in vitro more than sevoflurane-opioid anesthesia (27). Ruxanda et.al. 2016 in their comparative study on the effect of isoflurane and sevoflurane on rats' liver, revealed that isoflurane is superior to sevoflurane in the induction of apoptosis (28). However, loop et.al. reported that sevoflurane and isoflurane induce apoptosis in lymphocytes via increasing mitochondrial membrane permeability and caspase-3 activation, but independently of death receptor signaling (29). Wei et.al reported that prolonged exposure of rats to Isoflurane at 2.4% induced cytotoxicity, which was characterized by nuclear condensation and fragmentation and activation of caspases 3 and 9.

Moreover, Isoflurane reduced the Bcl-2/Bax ratio by 36%. However, sevoflurane did not cause neuronal damage by apoptosis nor did it decrease the Bcl-2/Bax ratio. They suggest that isoflurane and sevoflurane differentially affect the Bcl-2/Bax ratio (30).

Furthermore, Chen et.al. suggested that exposure to a sub-anesthetic dose of sevoflurane enhances hippocampal neurogenesis in neonatal rats and promotes their performance in dentate gyrus-dependent learning tasks (31). Immunofluorescence studies showed that sevoflurane exposure induces expression of cleaved caspase-3 and reduces expression of nNOS in neonatal rat hippocampus. Caspase-3, when activated by proteolytic cleavage, is one of the apoptotic effectors responsible for the breakdown of cellular components (32, 33).

To the best of our knowledge, we could not trace literature that illustrates the apoptosis process (apoptotic and antiapoptotic) during congenital heart

disease surgery. Wherever this study revealed that both apoptotic and antiapoptotic markers are active during surgery. Moreover, the activity increases with surgery duration. So, both intrinsic and extrinsic pathways are involved in the story to save good cells without neither necrosis nor proliferation.

The present study revealed that stress associated with an increase in free radicals in addition to many cytokines released mainly tumor necrosis factor-alpha and interleukins together with drugs used in anesthesia played a big role in the apoptotic pathway in these patients manifested by the significant increase in the three parameters studied. On the other hand, these phenomena may be beneficial in wound healing and postoperative inflammation. The present results also go hand in hand with the time of surgery, which means that induction of apoptosis is stimulated by different factors associated with stress and time-related factors. Besides, irreversible pulmonary hypertension may be associated with impaired apoptosis and antiapoptotic signaling from perivascular inflammatory cells.

Conclusion

Both apoptotic and antiapoptotic pathways are activated during congenital heart corrective surgeries. TF repair surgery, which has a wider incision line and is more complex than ASD and VSD repair, has resulted in a more severe increase in the level of apoptosis markers.

Acknowledgment

We would like to acknowledge the teamwork of the Metabolic and Genetic Disorders Unit (ISO 15189) Assiut University, where the laboratory work of this study has been done.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1. Brantley SJ, Cotten SW, Lamson DR, Smith GR, Liu R, Williams KP. Discovery of small molecule inhibitors for the C. elegans caspase CED-3 by high-throughput screening. Biochem Biophys Res Commun. 2017;491(3):773-9.
2. Chen X, Duan N, Zhang C, Zhang W. Survivin and Tumorigenesis: Molecular Mechanisms and Therapeutic Strategies. J Cancer. 2016;7(3):314-23.
3. Kim NH, Kang PM. Apoptosis in cardiovascular diseases: mechanism and clinical implications. Korean Circ J. 2010;40(7):299-305.
4. Zhang XP, Liu F, Wang W. Regulation of the DNA damage response by p53 cofactors. Biophys J. 2012;102(10):2251-60.
5. Kovacević M, Simić O, Jonjić N, Stifter S. Apoptosis and cardiopulmonary bypass. J Card Surg. 2007;22(2):129-34.
6. Zhou X, Li W, Chen X, Yang X, Zhou Z, Lu D, et al. Dose-dependent effects of sevoflurane exposure during early lifetime on apoptosis in hippocampus and neurocognitive outcomes in Sprague-Dawley rats. Int J Physiol Pathophysiol Pharmacol. 2016;8(3):111-9.
7. Wang H, Jiao H, Jiang Z, Chen R. Propofol inhibits migration and induces apoptosis of pancreatic cancer PANC-1 cells through miR-34a-mediated E-cadherin and LOC285194 signals. Bioengineered. 2020;11(1):510-21.
8. Siddiqui RA, Zerouga M, Wu M, Castillo A, Harvey K, Zaloga GP, et al. Anticancer properties of propofol-docosahexaenoate and propofol-eicosapentaenoate on breast cancer cells. Breast Cancer Res. 2005;7(5):R645-54.
9. Tsuchiya M, Asada A, Arita K, Utsumi T, Yoshida T, Sato EF, et al. Induction and mechanism of apoptotic cell death by propofol in HL-60 cells. Acta Anaesthesiol Scand. 2002;46(9):1068-74.
10. Engelhard K, Werner C, Eberspächer E, Pape M, Blobner M, Hutzler P, et al. Sevoflurane and propofol influence the expression of apoptosis-regulating proteins after cerebral ischaemia and reperfusion in rats. Eur J Anaesthesiol. 2004;21(7):530-7.
11. Liu KX, Chen SQ, Huang WQ, Li YS, Irwin MG, Xia Z. Propofol pretreatment reduces ceramide production and attenuates intestinal mucosal apoptosis induced by intestinal ischemia/reperfusion in rats. Anesth Analg. 2008;107(6):1884-91.
12. Roy N, Friehs I, Cowan DB, Zurakowski D, McGowan FX, del Nido PJ. Dopamine induces postischemic cardiomyocyte apoptosis in vivo: an effect ameliorated by propofol. Ann Thorac Surg. 2006;82(6):2192-9.
13. Acquaviva R, Campisi A, Raciti G, Avola R, Barcellona ML, Vanella L, et al. Propofol inhibits caspase-3 in astroglial cells: role of heme oxygenase-1. Curr Neurovasc Res. 2005;2(2):141-8.
14. Clark M, Choi J, Douglas M. Control of the cell cycle. Biology 2e. 2018.
15. Lévy M, Maurey C, Celermajer DS, Vouhé PR, Danel C, Bonnet D, et al. Impaired apoptosis of pulmonary endothelial cells is associated with intimal proliferation and irreversibility of pulmonary hypertension in congenital heart disease. J Am Coll Cardiol. 2007;49(7):803-10.
16. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35(4):495-516.
17. Dong Y, Chen H, Gao J, Liu Y, Li J, Wang J. Molecular machinery and interplay of apoptosis and autophagy in coronary heart disease. J Mol Cell Cardiol. 2019;136:27-41.
18. Nicholson DW. Caspase structure, proteolytic substrates, and

function during apoptotic cell death. Cell Death Differ. 1999;6(11):1028-42.
19. Venero JL, Burguillos MA, Joseph B. Caspases playing in the field of neuroinflammation: old and new players. Dev Neurosci. 2013;35(2-3):88-101.
20. Warren CFA, Wong-Brown MW, Bowden NA. BCL-2 family isoforms in apoptosis and cancer. Cell Death Dis. 2019;10(3):177.
21. Kumar R, Herbert PE, Warrens AN. An introduction to death receptors in apoptosis. Int J Surg. 2005;3(4):268-77.
22. Norton JA, Barie PS, Randal Bollinger R, Chang AE, Lowry SF, Mulvihill SJ, et al. Surgery: Basic Science and Clinical Evidence. 2nd ed. SpringerNature. 2008.
23. Delogu G, Moretti S, Antonucci A, Marcellini S, Masciangelo R, Famularo G, et al. Apoptosis and surgical trauma: dysregulated expression of death and survival factors on peripheral lymphocytes. Arch Surg. 2000;135(10):1141-7.
24. Xing SG, Zhang KJ, Qu JH, Ren YD, Luan Q. Propofol induces apoptosis of non-small cell lung cancer cells via ERK1/2-dependent upregulation of PUMA. Eur Rev Med Pharmacol Sci. 2018;22(13):4341-9.
25. Berns M, Wolter AC, Bührer C, Endesfelder S, Kerner T. Isoflurane but not Fentanyl Causes Apoptosis in Immature Primary Neuronal Cells. Open Anesth J. 2017;11(1):39-47.
26. Schenning KJ, Noguchi KK, Martin LD, Manzella FM, Cabrera OH, Dissen GA, et al. Isoflurane exposure leads to apoptosis of neurons and oligodendrocytes in 20- and 40-day old rhesus macaques. Neurotoxicol Teratol. 2017;60:63-8.
27. Xu YJ, Li SY, Cheng Q, Chen WK, Wang SL, Ren Y, et al. Effects of anaesthesia on proliferation, invasion and apoptosis of LoVo colon cancer cells in vitro. Anaesthesia. 2016;71(2):147-54.
28. Ruxanda F, Gal AF, Raţiu C, Miclăuş V, Rus V, Oana LI. Comparative immunohistochemical assessment of the effect of repetitive anesthesia with isoflurane and sevoflurane on rat liver. Braz J Anesthesiol. 2016;66(5):465-9.
29. Loop T, Dovi-Akue D, Frick M, Roesslein M, Egger L, Humar M, et al. Volatile anesthetics induce caspase-dependent, mitochondria-mediated apoptosis in human T lymphocytes in vitro. Anesthesiology. 2005;102(6):1147-57.
30. Wei H, Kang B, Wei W, Liang G, Meng QC, Li Y, et al. Isoflurane and sevoflurane affect cell survival and BCL-2/BAX ratio differently. Brain Res. 2005;1037(1-2):139-47.
31. Chen C, Shen FY, Zhao X, Zhou T, Xu DJ, Wang ZR, et al. Low-dose sevoflurane promotes hippocampal neurogenesis and facilitates the development of dentate gyrus-dependent learning in neonatal rats. ASN Neuro. 2015;7(2).
32. Young C, Roth KA, Klocke BJ, West T, Holtzman DM, Labruyere J, et al. Role of caspase-3 in ethanol-induced developmental neurodegeneration. Neurobiol Dis. 2005;20(2):608-14.
33. Zhou X, Song FH, He W, Yang XY, Zhou ZB, Feng X, et al. Neonatal exposure to sevoflurane causes apoptosis and reduces nNOS protein expression in rat hippocampus. Mol Med Rep. 2012;6(3):543-6.