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RESEARCH ARTICLE

NGS of microRNAs Involved in Cardioprotection Induced by Sevoflurane Compared to Propofol in Myocardial Revascularization Surgery: The ACDHUVV-16 Clinical Trial

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Abstract: ***Background*:** Numerous studies have demonstrated that halogenated agents elicit myocardial conditioning effects when administered perioperatively in cardiac surgery. Recent evidence has been published on the benefits of maintaining exposure to halogenated agents during the early postoperative period. The enzymatic mechanisms by which this beneficial effect is exerted were explained recently.

***Objectives*:** Our study was performed to investigate whether this phenomenon is mediated by either the activation or suppression of miRNAs targeted by halogenated anesthetics.

***Methods*:** A double-blind, two-stage trial was conducted. The results of the first stage of the trial are presented in this paper. The sample was composed of patients undergoing off-pump myocardial revascularization surgery. Patients were randomized to receive either sevoflurane [S] or propofol [P] during the intraoperative and early postoperative period (during the first six hours after the intervention). Hemodynamics (heart rate, blood pressure, central venous pressure, cardiac index, systolic volume index, LVEF) and myocardial enzymes (troponin I) were monitored at six hour intervals during the first 48 hours. In the first stage of the trial, blood was drawn for gene sequencing from eight patients (four per group) at baseline and at 24 h. In the second stage of the study, a qPCR analysis was performed of the miRNAs identified as significant by gene sequencing. Levels of cardioprotective enzymes (serine/threonine protein kinase (Akt), tumor necrosis factor alpha (TNFα), extracellular regulated protein kinase (ERK 1/2), and caspase 3) were measured to assess their role in myocardial conditioning pathways. The purpose was to identify the miRNAs that play a major role in myocardial conditioning induced by halogenated agents. Concentrations of cardioprotective enzymes were higher in patients who received sevoflurane than the patients who were administered propofol.

***Results*:** NGS differences were observed between baseline and 24-h values in the two study groups. In group P, miRNA 197-3p was overexpressed, whereas miRNAs 4443 and 1294, 708-3p were underexpressed. In group S, miRNAs 615-3p, 4466, 29, 937-3p, 636, 197-3P, 184, 4685, 296-3p, 147b, 3199, 6815, 1294 and 3176 were underexpressed; whereas 708-3p was overexpressed. qPCR showed significant variations in miRNAs 197-3p, 4443, 708-3p and 1294 in the P group, and in miRNAs 937-3p, 636, 197-3p, 296-3p and 708-3p in the S group.

***Conclusions*:** In the P Group, changes in the expression of some miRNAs were associated with lower concentrations of the enzymes involved in myocardial pre- and postconditioning. In contrast, in Group S, variations in miRNAs were associated with the activation of mediators of anesthetic-induced pre- and post-conditioning, a reduction in cell apoptosis, and a decrease in caspase and TnBF alpha concentrations. Changes in these miRNAs were associated with better prognosis in patients with ischemic heart disease. The main limitation of this study will be overcome in the second stage of the trial, where the specific role of each miRNA will be determined.

Keywords: miRNAs, cardiac anesthesia, preconditioning, postconditioning, aconditioning, halogenated.

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1. INTRODUCTION

The effects of hypnotics on the myocardium of cardiac surgery patients have been addressed in numerous studies, with differing objectives and results [1]. The cardioprotective effects of halogenated agents (vs intravenous anesthetics) administered perioperatively in cardiac surgery depend on exposure time. Thus, a longer exposure to halogenated agents at standard concentrations routinely used in clinical practice exerts greater clinical benefits [2-4].

Myocardial conditioning is the mechanism by which cardioprotection is elicited. This effect includes cardiac pre- and postconditioning, which are induced by exposure to a halogenated agent prior to ischemia/reperfusion [1]. Myocardial conditioning has been mostly studied in patients undergoing myocardial revascularization surgery [5-7]. This effect reduces ischemic/reperfusion injury, results in lower use of inotropic drugs, helps preserve renal function, and limits the length of ICU stay [8]. Conflicting results have been obtained in terms of mortality in cardiac surgery patients due to the agent used [9, 10].

Myocardial pre- and postconditioning are triggered by the activation of several enzymatic pathways, including, but not limited to, the RISK and SAFE pathways. The activation of these pathways reduces cellular apoptosis and cardiac stunning [11]. Some of the proteins involved in these pathways have been documented to be overexpressed in cardiac surgery patients. Differences increase when a halogenated agent is administered intra- and postoperatively as a hypnotic.

Micro RNAs (miRNAs) are molecules containing nucleotides with the ability to inhibit specific MRNAs that either stimulate or inhibit the synthesis of some enzymes. Some of these enzymes are involved in ischemic myocardial conditioning pathways [12]. However, the mechanism of action and influence of hypnotics on gene expression during perioperative pre- and postconditioning are poorly understood.

The objective of this study was to explore the influence of propofol and sevoflurane on miRNA expression by complete next-generation sequencing. The first stage of the clinical trial involved patients undergoing off-pump myocardial revascularization surgery. Patients were administered anesthesia and sedation either with sevoflurane or propofol in the immediate perioperative period. The purpose was to assess variations in the expression of a panel of miRNAs 24 hours after surgery and 12 hours after exposure with respect to baseline expression. mRNA expression was assessed by massive parallel sequencing. As variations were detected in mRNA expression, we further investigated their role in the modulation of mediators of pre- and postconditioning induced by halogenated agents.

2. MATERIALS AND METHODS

A prospective, randomized, controlled, double-blind, phase IV clinical trial was conducted in Virgen de la Victoria University Hospital, Spain. The study was approved by the local Ethics Committee and the institutional review board of our hospital (CEI Malaga Norte Ethics Committee-random trial registration number: 1406; 16 December 2016). Patients were recruited and informed consent was obtained according to the applicable guidelines and regulations. Inclusion criteria were: age $\geq$ 18 years; elective off-pump myocardial revascularization surgery; EUROSCORE II < 3% (a European scientifically-validated risk score used during the perioperative period of cardiac surgery) (low-moderate cardiologic risk in the perioperative period); and an ASA operative risk score < 4 (patient with a moderate-high operative risk). Exclusion criteria were: history of adverse reaction to anesthetics; known severe organ impairment (lung, liver, kidney); combined surgery (valve repair or carotideal surgery); hemodynamic instability; heart failure or need for inotropics or vasoactive agents prior to the intervention; use of oral antidiabetics not suspended at least 48 hours prior to the intervention; eufilin / theophylline therapy prior to the intervention; inability to provide informed consent; pregnancy or breastfeeding.

During the first stage of this trial, variations in mRNAs 24 hours after surgery with respect to baseline values were assessed in eight patients. Randomization was performed using Epidat 4.0 ® (Sergas, Galicia, Spain) software package. Patients were allocated to one of these groups:

PP Group: Anesthesia with propofol + sedation with propofol.

SS Group: Anesthesia with sevoflurane + sedation with sevoflurane.

2.1. Anesthesia and Surgical Procedure

Intraoperative monitoring included continuous 5-lead electrocardiogram recording leads II and V; invasive arterial pressure recorded from the radial artery; cardiac output determined via a pulmonary artery line; pulse oximetry; capnography; hypnosis by bispectral index (BIS) (BIS XP®, Aspect Medical Systems, Newton, MA); pharyngeal, bladder and blood temperature.

The PP group was administered a bolus of fentanyl 4mcg/kg and received continuous target-controlled infusion (TCI) of propofol (Diprivan®; AstraZeneca, Brussels, Belgium) for a plasma concentration of 2 mcg/mL adjusted according to the BIS value. In the SS group, anesthesia was induced through a bolus injection of etomidate 0.3 mg / kg and fentanyl 4 mcg/kg. Anesthesia was maintained with sevoflurane (Sevorane®; Abbott, Louvain-la- Neuve, Belgium) adjusted according to the BIS value.

To ensure adequate hypnosis and prevent overdosing, infusion rates were adjusted to maintain BIS within the range of 45-60. Surgery was performed according to standard protocols. Surgical preconditioning was not induced in any patient. Upon completion of the intervention, patients were transferred to the ICU under sedation with either propofol or sevoflurane until extubation. Six hours after admission, if criteria were met, extubation was carried out in accordance with the standard protocol. In the ICU, sedation with propofol was maintained at plasma concentrations of 1.5mcg/mL in the PP group via TCI infusion to keep hypnosis values between 60 and 70. In the SS group, sedation was induced with sevoflurane using the Anaesthetic Conserving Device AnaConDa© (ACD; Sedana Medical, Uppsala, Sweden), which corresponds to an end-tidal concentration of sevoflurane of 0.5%-0.7%.

2.2. Hemodynamic Monitoring

cardiac output was monitored using a continuous cardiac output monitor (MostCare Up®; Vygon, Ecouen, France) system recording cardiac index, central venous saturation, and systolic volume index. Other hemodynamic variables including heart rate, mean blood pressure, and right-ventricular end-diastolic pressure were monitored before the induction of general anesthesia (baseline), following anesthesia, intubation and coronary bypass surgery, and 48 hours after the start of the intervention.

2.3. Biochemistry

Troponin I was determined after anesthesia induction and intubation, and at 6 hours, 12 hours, 24 hours and 48 hours after the intervention had started. NT-pro BNP was determined at baseline and at 24 hours.

2.4. Enzymes Exerting Beneficial Effects

Blood was drawn from the median ulnar vein and placed in vacutainer tubes (BD vacutainer, London, UK). Prior to surgery and at 24 postoperative hours, serum was separated by centrifugation for 15min at 4000 rpm and immediately frozen at −80 °C until immunoassay analysis (serine/threonine protein kinase (Akt), tumor necrosis factor alpha (TNFα), and extracellular-regulated protein kinase (ERK 1/2), and caspase 3).

2.5. Massive sequencing method

2.5.1. Massive RNA (miRNA) Sequencing

miRNA was extracted using an extraction kit (miRCURYTM RNA Isolation Kit- tissue and biofluids; Exiqon). An aliquote was used for miRNA library sequencing (Centro de Supercomputación y Bioinnovación, University of Malaga) to study miRNA (n=8) expression patterns. The other aliquote was used for confirmation of rt-PCR results. cDNA was obtained using the Universal cDNA Synthesis (Exiqon) kit. The quality of cDNA extraction and RT was assessed using a QC panel (miRCURY LNATM Universal RT miRNA PCR, 16 Ready-to-Use miRNA QC PCR Panels) in an Applied Biosystems Fast 7500 ((miRCURY L ATM Universal RT miRNA PCR, with synthetic RNA spike-in templates for qPCR control (UniSp2, UniSp4, UniSp5 RNA Spike-in template mix and cel-miR-39-3p RNA Spike-in template. The efficacy of extraction was assessed by Spike-in UniSp2, UniSp4 UniSp5; cDNA synthesis by spike-in cel-39-3p and UniSp6; and PCR reaction was evaluated by spike-in UniSp3).

2.5.2. RNA Quality for Ultrasequencing

Once miRNA was extracted, RNA was analyzed prior to sequencing to assure that it met specific qualitative and quantitative standards. miRNA quality was analyzed by lab-on-a-chip electrophoresis using the bioanalyzer 2100 kit (Agilent). Quantification of samples was performed using a Qubit (Life Technologies) fluorimeter (LifeTechnologies). Aliquotes were stored at -80 ºC for later processing.

Once miRNA libraries were prepared, sequencing was performed using NextSeq 550 (Illumina). This sequencing platform is based on Illumina sequencing-by-synthesis (SBS) technology, with two-run modes: the High mode, which generates 400 million simple reads (800 million parallel reads), and the Mid run mode, which generates 130 million simple reads (260 million parallel reads). This system enables the sequencing of around 30 miRNA samples per run (High) for a minimum of 5-10 million reads per sample. 1%-PhiX was used as a control in each run to verify the quality of clustering and sequencing and determine the alignment error rate. In total, 75% of reads obtained by this tech-

nique have a quality index >Q30. Simple 1x 50-75pb miRNA sequencing was performed.

2.5.3. Experimental Validation of miRNA Expression by RT-qPCR

The expression of eight miRNAs was assessed in the same samples (n=4 per group) by RT-qPCR (technical validation) using the TaqMan® MicroRNA Reverse Transcription kit following manufacturer's instructions (ThermoFisher Scientific Inc, Waltham, MA). miRNA expression was determined by real-time PCR using the Applied Biosystems 7500 Fast Real-Time polymerase chain reaction System (Applied Biosystems, Foster City, CA). Reactions were carried out in duplicate for all miRNAs using TaqMan™ MicroRNAassay (Thermo Fisher Scientific Inc, Waltham, MA): hsa-miR-197-3p (Assay ID: 477959_mir), hsa-miR-708-3p (Assay ID: 479162_mir), hsa-miR-636 (Assay ID: 478185_mir), hsa-miR-4685-3p (Assay ID: 479921_mir), hsa-miR-296-3p (Assay ID: 478790_mir), hsa-miR-3199 (Assay ID: 479674_mir), hsa-miR-1294 (Assay ID: 478693_mir), hsa-miR-3176 (Assay ID: 478016_mir), hsa-miR-4443 (Assay ID: 479427_mir), hsa-miR-615-3p (Assay ID: 478175_mir), hsa-miR-4466 (Assay ID: 483160_mir), hsa-miR-29 (Assay ID: 478587_mir (hsa-miR-29a-3p)), hsa-miR-937-3p (Assay ID: 479212_mir), hsa-miR-184 (Assay ID: 477938_mir), hsa-miR-147b (Assay ID: 483120_mir (hsa-miR-147b-5p)) and hsa-miR-6815 (Assay ID: 480396_mir (hsa-miR-6815-5p)). The threshold cycle (Ct) value for each sample was normalized with the expression of hsa-miR-126-5p (Assay ID: 477888_mir). SDS software 2.3 and RQ Manager 1.2 (AppliedBiosystems, Foster City, CA) were used to analyze results by the comparative Ct method (2-DCt).

2.6. Data Analysis

2.6.1. miRNA Expression was Analyzed by RNA-sequencing

Bioinformatics were used to determine the miRNAs that were differentially expressed in each group. Data analysis was performed in several stages, namely:

1. First, read data preprocessing was performed to remove low-quality adapter nucleotides and delete those exposed to a source of contamination. In this stage, we employed SeqTrimNext, a software package developed by the Andalusian Platform of Bioinformatics. This tool performs parallel, distributed processing of large datasets as those generated by NGS.

2. Secondly, read alignment to the last version of human genome (hg38) was carried out using TopHat pipeline. This tool has the ability to recognize exon-intron processing sites and was optimized to identify gene isoforms and discover new genes. Expression level was reported as RPKM (reads per kilo base per million mapped reads). miRNA reads were aligned to reference reads, in this case, the last version of miRNA databases (http://www.mirbase.org/) using TopHat. TopHat was optimized for alignment to both the mature and immature form of the miRNA panel. Expression level was reported as the number of mapped reads.

3. In the third stage, the different datasets were compared to identify genes showing differential expression. This process involved previous normalization of gene expression data and dataset comparison.

4. The final result was a panel of genes or differently-expressed miRNAs that met requirements such as stability across the population, minimum level of expression, and statistical significance, among others.

Small RNA-seq for these assays is publicly available in the NCBI Sequence Read Archive (SRA) under accession number NCBI PRJNA594742.

2.7. Analysis of Other Data

We analyzed the remainder of data by standard descriptive statistics (*i.e.* Student's *t*-test, Fisher's test, or Man-Whitney *U* test for comparison of groups). Comparison of more than two groups was performed by ANOVA or Kruskal-Wallis. Correlations between variables were assessed by Spearman's test. Multiple linear regression analysis was performed to explore the presence or absence of colinearity among variables and determine the validity of bivariate correlations. The level of rejection of the null hypothesis was 0.05. All data are expressed as mean values ± standard deviations, unless otherwise stated.

3. RESULTS

No significant differences were observed in terms of epidemiological data, preoperative risk, or number of coronary bypass interventions (Table **1**).

Hemodynamics (heart rate, blood pressure, central venous pressure, cardiac index, systolic volume index, LVEF) and myocardial enzymes (troponin I) were monitored during the first 48 hours (Table **2**).

Patients who received sevoflurane exhibited higher concentrations of cardioprotective enzymes, as compared to patients who received propofol (serine/threonine protein kinase (Akt), tumor necrosis factor alpha (TNFα), extracellular-regulated protein kinase (ERK 1/2), caspase 3) (Figs. **1** and **2**).

Table 1. Patient characteristics.

	SS	PP
Sex (M/F)	2/2	2/2
Age	64-74	61-72
Size (cm)	156-170	164-171
EUROSCORE II	1.22%	1.43%
ASA class	III(II-IV)	III(II-IV)
EjectionFraction(%)	54%+/-8%	57%+/-7%
Number of by-passes	2:1	2:2
(1,2 or 3)	3:3	3:2
Reconversion CPB	0	0
Preoperative treatment and comorbidities:		
Hypercholesterolemia/statins	4	4
Hypertension/B-Blockers	4	4
Hypertension/ACE inhibitors	4	4
Ischemich heart disease/Nitrates	4	4
Hypertension/Calcium antagonist blockers	0	1
Heart Failure/Diuretics	0	0
Respiratory disease/Bronchodilating agents	1	1
Ischemich heart disease /Acetyl salicylic acid	4	4
Insulin	2	2

Data are expressed as absolute values, medians and ranges, as appropriate. There were no statistically significant differences between the two groups.
ASA: American Society of Anesthesiologists (physical status).
CPB: Cardiopulmonary by-pass.
SS: Intra and Postoperative Sevoflurane
SP: Intraoperative Sevoflurane and Postoperative Propofol
PP: Intra and Postoperative Propofol

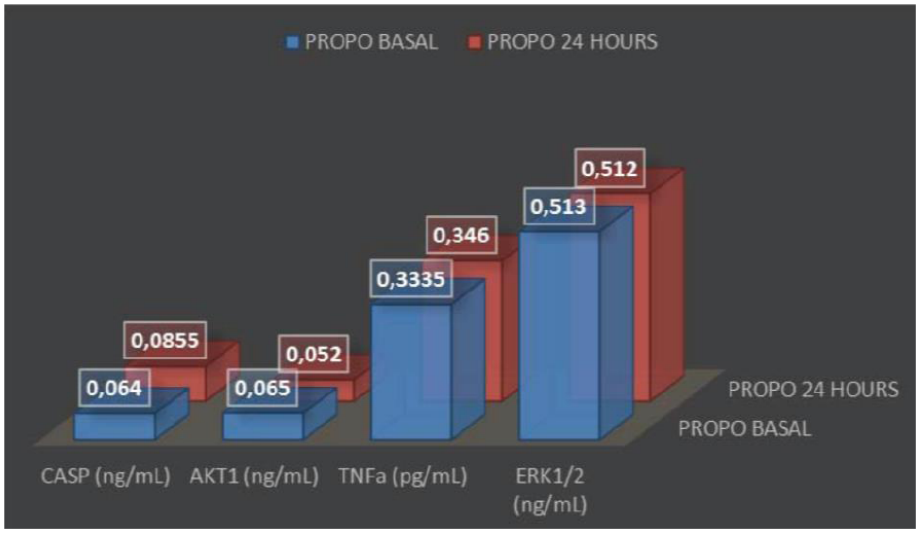

Fig. (1). Concentrations of enzymes involved in pre and postconditioning: propofol group. Concentrations of these enzymes were higher in patients who received sevoflurane as compared to propofol (serine/threonine protein kinase (Akt), tumoral necrosis factor alfa (TNFα), extra-cellular regulated protein kinase (ERK 1/2), caspase 3). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

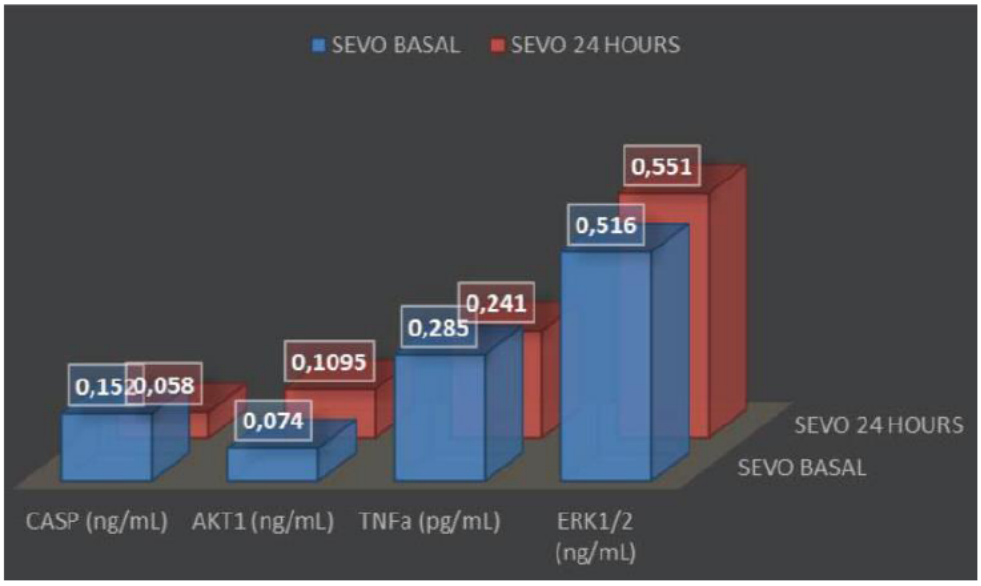

Fig. (2). Concentrations of enzymes involved in pre- and postconditioning: sevoflurane. Concentrations of these enzymes were higher in patients who received sevoflurane as compared to propofol (serine/threonine protein kinase (Akt), tumoral necrosis factor alfa (TNFα), extra-cellular regulated protein kinase (ERK 1/2), caspase 3). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Blood tests were performed in eight patients (four from each group) at baseline and 24 h for Next-generation Sequencing (NGS). Sequencing of 16 samples was performed. In a second stage, qPCR analysis was performed of the miRNAs identified as significant by gene sequencing. The purpose was to determine the miRNAs with a major role in cardioprotection induced by halogenated drugs. NGS differences were observed between baseline and 24-h values in the two study groups. In group P, miRNAs 197-3p was overexpressed, whereas miRNAs 4443 and 1294, 708-3p were underexpressed. In group S, miRNAs 615-3p, 4466, 29, 937-3p, 636, 197-3P, 184, 4685, 296-3p, 147b, 3199, 6815, 1294 and 3176 were underexpressed; and 708-3p was overexpressed.

The qPCR technique was used to valídate NGS results for all miRNAs with variations in the two groups. qPCR showed significant variations ($p<0.05$) in miRNAs 197-3p, 4443, 708-3p and 1294 in the P group. qPCR showed significant variations ($p<0.05$) in miRNAs 937-3p, 636, 197-3p, 296-3p and 708-3p in the S group (Figs. **3** and **4**).

4. DISCUSSION

Compared to propofol in the perioperative period of myocardial revascularization surgery, the use of halogenated agents reduces myocardial injury. This effect is modulated by the activation of enzymatic mediators of myocardial pre- and postconditioning (11). In our study, complete parallel sequencing revealed that some miRNAs are associated with the cardioprotective effects of halogenated agents, as compared to intravenous agents. In our study, the miRNAs that mediate this effect were found to be different from those involved in ischemic conditioning.

In the P Group, changes in the expression of miRNAs were associated with a decrease in the levels of enzymes involved in myocardial pre- and postconditioning. The same phenomenon was observed in mediators of atheromatous disease progression. In contrast, in Group S, variations in miRNAs correlated with the activation of mediators of anesthetic-induced pre- and post-conditioning, decreased cell apoptosis, and a reduction in caspase TnBF alpha concentrations. Additionally, changes in these miRNAs were associated with better prognosis in patients with ischemic heart disease.

miRNA 197-3 was overexpressed when propofol was used as a hypnotic. This finding has been related to PI3K and AKT modulation, which are key mediators of myocardial preconditioning. Thus, the upregulation of PI3K and AKT triggers the inhibition of protein pathways involved in myocardial protection. Notably, 708-3p was underexpressed in the propofol group and overexpressed in the other treatment group. Downregulation of this miRNA reduces Akt and JNK MAP kinase

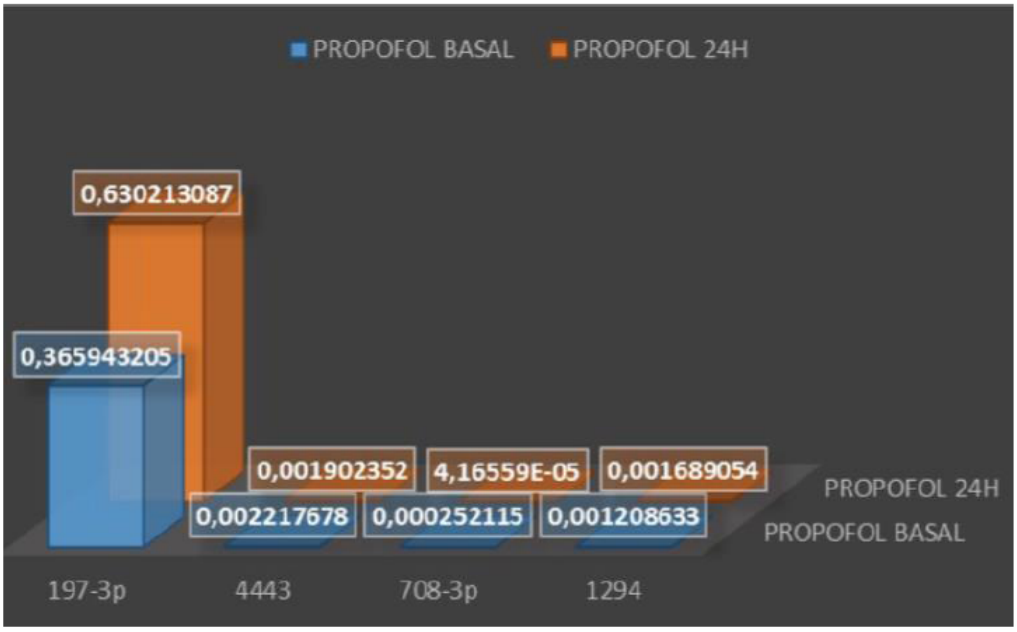

Fig. (3). Significant differences were observed between baseline and 24-h values in propofol study group. In group P, miRNAs 197-3p was overexpressed in group P, while miRNAs 4443 and 1294, 708-3p were underexpressed. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

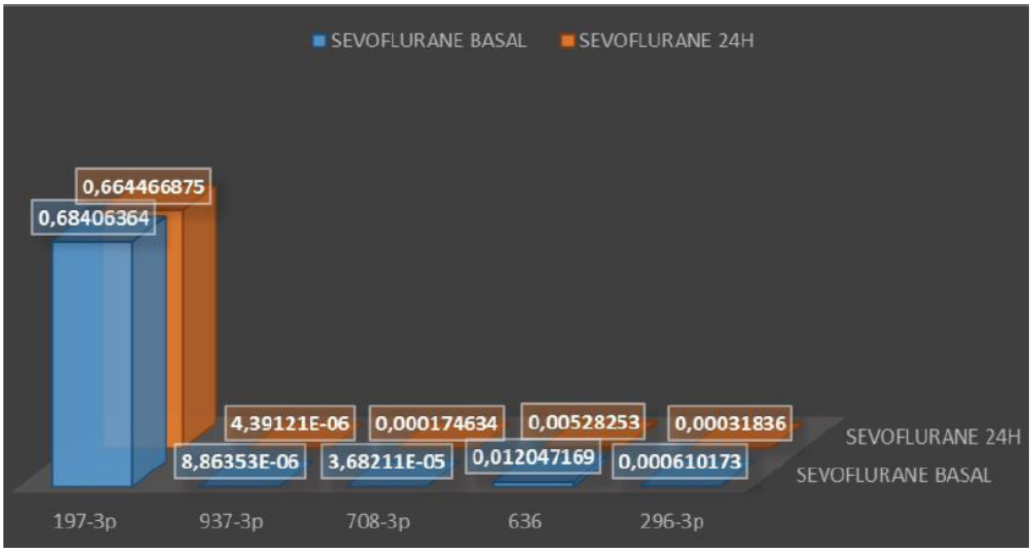

Fig. (4). Significant differences were observed between baseline and 24-h values in propofol study group. miRNAs 937-3p,636 , 197 3p, 296 3p were underexpressed and 708-3p was overexpressed in the S group. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

levels, which are essential to myocardial conditioning [13, 14].

In contrast, exposure to propofol induced a reduction in miRNA 4443. Increased concentrations of this miRNA drive JAK2/STAT3 overexpression, which plays an essential role in the mechanisms of drug-induced myocardial conditioning [15]. Finally, miRNA 1275 was underexpressed in the propofol group [16]. The role of this miRNA in myocardial preconditioning seems to be mediated by its effects on mitochondria and the activation of the molecular process that confers cardioprotection. miRNA 1275 is involved in myocardial apoptosis via ATP breakdown products (AMP, adenosine and monophosphate inosine). These products trigger AMPK activation, which generates a hypometabolic state by mitochondrial and K-channel preservation. K-channels are crucial to myocardial cell survival

mediated by drug-induced myocardial conditioning. Therefore, downregulation of the miRNAs with a major role in drug-induced myocardial preconditioning and mitochondrial preservation will result in the inactivation of the signaling pathways involved in cardioprotection [17].

Substantially different results were obtained in the sevoflurane group. Thus, in group S miRNAs 615-3p, 4466, 29, 937-3p, 636, 197-3P, 184, 4685, 296-3p, 147b, 3199, 6815, 1294 and 3176 decreased and 708-3p was overexpressed. However, subsequent qPCR only confirmed significant variations in miRNAs 937-3p, 636, 197-3p, 296-3p, which were underexpressed, and in 708-3p, which was overexpressed.

Although their expression is not confirmed by qPCR, the MiRNAs 4466, 29, 4685, 184, 1294, 615-3p, 147b, 6815 and 3176 are involved in myocardial pre- and post-conditioning pathways. Therefore, the modulation of these miRNAs may have cardioprotective effects [18-28].

miRNA 708-3p overexpression induced by exposure to sevoflurane results in the upregulation of Akt and JNK MAP kinase [14]. miRNA 937-3p overexpression causes the inhibition of PI3K/Akt, which are related to preconditioning. Lower concentrations of miRNA 937-3p were observed at 24 hours, which may be related to an increase in PI3K/Akt levels [22]. Evidence has been published showing a relationship between miRNA 636 underexpression and myocardial injury secondary to acute myocardial infarction [23]. miRNA 296-3p levels reportedly decrease after exposure to sevoflurane [29]. These miRNAs play a major role in the modulation of NF-κB, which is involved in cellular response to stimuli such as stress and the inhibition of STAT protein response. In some settings, NF-κB favors cellular apoptosis. Therefore, NF-κB downregulation could create a favorable environment for the activation of myocardial conditioning mechanisms [30].

Of the miRNAs variations were observed, the miRNA which expression changed most significantly was miRNA-197-3p. In previous studies, miRNA-197-3p overexpression has been associated with coronary ischemia, as it increases notably in the presence of myocardial ischemia, regardless of whether the affected region is the right or the left ventricle. Consequently, miRNA-197-3p has been proposed as a useful marker in the setting of ischemic heart disease. miRNA-197 promotes platelet activation and has been associated with risk factors of coronary artery disease such as dyslipidemia and metabolic syndrome. It has also been strongly related to cardiometabolic disease. In the context of tissue hypoxia and reduced coronary blood flow, this miRNA is overexpressed in the cardiomyocyte. miRNA-197 belongs to the Hypoxiamir group of miRNAs, which are involved in the development of ischemic heart disease [11, 15, 20-25].

A recent study related elevated levels of miRNA-197 in plasma with increased mortality from acute coronary syndrome, which points at this miRNA as a very useful diagnostic, prognostic and therapeutic marker in ischemic heart disease.

Our study revealed that miRNA 197-3p concentrations increased after patients were exposed to propofol, which did not occur in those treated with sevoflurane. In our opinion, this is the main finding of our study. The clinical benefit exerted by sevoflurane in the perioperative period after myocardial revascularization surgery in patients with coronary artery disease may be mediated by variations in miRNA expression.

In a later stage, we investigated the relationship between miRNA overexpression and the different affected genes using the tool available at www.minitargets.com. Although these genes were not directly determined during the study, they may be the final receptors of the gene modulation sequence triggered by the different hypnotics (Table **3**). In our opinion, future studies are needed to demonstrate that the relationship described is consistent with our findings.

The inconsistency of our results as compared to those of previous studies may lie in methodological differences, as the expression of miRNAs associated with heart disease was assessed by arrays. Some of the miRNAs tested are known to play a role in non-cardiac diseases, whereas many others have not been found to be involved in ischemic pre- and postconditioning. In contrast, these miRNAs modulate the protein pathways that confer myocardial preconditioning induced after prolonged intra- and postoperative exposure to halogenated agents. To the best of our knowledge, this is the first study to identify the miRNAs which expression changes after exposure to propofol and sevoflurane in patients with ischemic heart disease undergoing off-pump myocardial revascularization surgery.

The results obtained confirm that differences between treatment groups are explained by the beneficial effects of anesthesia and postoperative sedation with sevoflurane on gene expression; differences are also induced by propofol's effects on the miRNAs that induce the downregulation of enzymatic mediators of myocardial conditioning.

Table 3. The target genes of specific miRNAs can be detected using labeled mRNA. This assay can be performed at www.mirnatargets.com.

	miRNA 197-3p	miRNA 708-3P	miRNA 4443	miRNA 1294	miRNA 937-3p	miRNA 636	miRNA 296-3p
TARGET GENES	PIK3R5, F730038I5Rik, FOAP-2, P101-PI3K and p101	MAPK1, ERK, ERK – 2, ERK2, ERT1, MAPK2, P42MAPK, PRKM1, PRKM2, p38, p40, p41, p41mapk, p42-MAPK	IFNAR1, AVP, IFN – alpha – REC, IFNAR, IFNBR, IFRC for miRNA 4443, and CSF3R, CD114, GCSFR	SCN7	NOS1	AKT3, MPPH, MPPH2, PKB-GAMMA, PKBG, PRKBG, RAC – PK – gamma, RAC – gamma, STK - 2.	CCND1, BCL1, D11S287E, PRAD1, U21B31

The results of the first stage of this clinical trial suggest that gene expression is modulated differently according to the type of hypnotic used. Thus, the level of cardioprotection conferred by halogenated agents depends on the genes modulated by each agent. The fact that sevoflurane confers myocardial conditioning effects is unquestionable. Several authors investigated the benefits of sevoflurane in coronary surgery, with inconsistent results. Guerrero *et al.* described the cardiac pre and post-conditioning mechanisms by which sevoflurane exerts clinical benefits when administered during surgery and during the first 6 postoperative hours, as compared to intraoperative administration only or propofol [2, 3, 11, 31, 32].

On the other hand, propofol modulates the expression of some miRNAs, thereby inhibiting many of the protective mechanisms activated against ischemic myocardial injury.

The main limitation of this study is that concentrations of the miRNAs involved in drug-induced myocardial conditioning were only determined in a small sample of patients. The second stage of this clinical trial will solve this limitation. Gene sequencing was performed in eight patients, a reasonable number, given the cost and complexity of this technique. Another limitation is that this is a single-center study. However, this allowed us to perform the study in patients who underwent off-pump myocardial revascularization surgery, which prevents the release of associated mediators. All interventions were performed by expert surgeons. Another limitation was major cardiovascular comorbidities such as hyperlipidemia, diabetes, and their co-medications, which could interfere with cardioprotective mechanisms. This potential interference should be taken into account [33].

CONCLUSION

Intra- and postoperative use of sevoflurane as a hypnotic vs propofol in patients undergoing off-pump myocardial revascularization surgery induces the modulation of multiple miRNAs involved in myocardial pre- and postconditioning, with a most significant effect on miRNA 197-3p. The miRNAs related to the cardioprotective effects of halogenated agents partially differ from those involved in the reduction of myocardial injury by ischemic conditioning.

LIST OF ABBREVIATIONS

S = Sevoflurane

P = Propofol

Akt = Serine/threonine protein kinase

TNFα = Tumor necrosis factor alpha

ERK 1/2 = Extracellular regulated protein kinase

ICU = Intensive Care Unit

miRNAs = Micro RNAs

BIS = Bispectral index

TCI = Continuous target-controlled infusion

AnaConDa© = Anaesthetic Conserving Device

NGS = Next-generation Sequencing

CLINICAL PERSPECTIVES

1. Background: Numerous studies have demonstrated that halogenated agents elicit a myocardial conditioning effect when administered perioperatively in cardiac surgery. There is recent evidence of the benefits of maintaining patient's exposure to halogenated agents during the early postoperative period. The enzymatic mechanisms by which this beneficial effect

occurs have been explained recently. We conducted a study to investigate whether this phenomenon is modulated by the activation or suppression of miRNAs specifically mediated by halogenated anesthetics.

2. A brief summary of the results: A double-blind, two-phase trial was conducted. We present the results of the first phase of the trial. The sample was composed of patients undergoing off-pump myocardial revascularization surgery. Patients were allocated to receive either sevoflurane (S) or propofol (P) during the intraoperative and early postoperative period (six hours after the intervention). Hemodynamics (heart rate, blood pressure, central venous pressure, cardiac index, systolic volume index, LVEF) and myocardial enzymes (troponin I, CPK 2) were assessed every six hours during the first 48 hours. In the first phase of the trial, blood tests were performed in eight patients (four from each group) at baseline and at 24 h for gene sequencing. In a second phase, a qPCR analysis of the miRNAs identified by gene sequencing as significant will be conducted to determine the miRNAs with a major role in halogenate-drug-induced myocardial conditioning.

Significant differences were observed between baseline and 24-h values in the two study groups.

3. The potential significance of the results to human health and disease: the expression of different miRNAs in the P Group was associated with a decrease in the expression of enzymes associated with myocardial pre- and postconditioning (STAT group, MAPK enzyme) and of mediators of atheromatous disease progression. In contrast, in Group S, variations in miRNAs correlated to the activation of anesthetic-induced pre- and post-conditioning mediators (AKT, ERK, PI3K/Akt pathways, STAT group), a reduction of cell apoptosis by p53 protein activation; and a reduction of caspase 3 and 6 and TBF alpha concentrations. Additionally, changes in these miRNAs correlated with increased survival in patients with ischemic heart disease and a reduced incidence of arrhythmia and atherosclerotic damage.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The clinical trial was monitored by the Instituto Biomédico de Málaga (IBIMA) and was conducted in accordance with GCP and European GDPR. This manuscript adheres to the applicable CONSORT guidelines. The clinical trial was conducted in Virgen de la Victoria University Hospital, Spain. The study was approved by the local Ethics Committee and the institutional review board of our hospital (CEIMalaga Norte Ethics Committee-random trial registration number: 1406; 16 December 2016)

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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