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Original article

MMP-9 and MMP-2 regulation in patients undergoing non-oncological and non-vascular elective surgery independent of the use of propofol or sevoflurane

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ABSTRACT

Background: There is debate regarding whether inhaled sevoflurane or intravenous propofol used during anesthesia achieves the best outcome. Propofol has been shown to affect expression of matrix metalloproteinases (MMPs). MMPs are enzymes that play a role in extracellular matrix remodeling, with activity balance disturbances during surgery. The goal of this study was to compare MMP-2/9 concentrations, activity, and tissue inhibitors of metalloproteinases (TIMPs) 1/2 concentrations in blood of who had undergone 2 types of anesthesia: based on volatile sevoflurane and intravenous propofol during non-oncological, non-vascular surgery.

Methods: 39 patients were enrolled into analysis, 20 anesthetized with total intravenous anesthesia with propofol (P), 19 with volatile induction/maintenance of anesthesia with sevoflurane (S). Plasma samples collected before and 24 h after surgery were analyzed for MMP-2/9, and TIMP-1/2 concentrations using ELISAs. Additionally, MMP-2/9 activities were assessed by gelatin zymography.

Results: Study revealed increased MMP-9 concentration (ELISA) (P:$p = 0.011$; S:$p = 0.001$) and activity (zymography) (P:$p = 0.004$; S:$p = 0.008$) in both groups 24 h after surgery. We noticed decreased (both groups) MMP-2 concentration (P:$p = 0.044$; S:$p = 0.027$) with MMP-2 activity increase (P:$p = 0.002$; S:$p = 0.006$) 24 h after surgery. We observed decreased TIMP-1 plasma concentrations (P:$p = 0.002$; S:$p = 0.000$) 24 h after procedures, while TIMP-2 plasma levels remain unchanged (P:$p = 0.097$; S:$p = 0.172$). There were no differences between concentration and activity of MMPs and TIMPs in regard to anesthetic used. Meperidine administration correlated with lower MMP-9 activity (R=-0.430; $p = 0.006$).

Conclusions: Concluding, neither sevoflurane nor propofol used as anesthetics modulate MMP-2 and MMP-9 concentrations and activities during non-oncological, non-vascular elective surgery. Meperidine seems to decrease MMP-9 activity.

Introduction

There is continuous debate regarding which anesthetic techniques and agents should be used for the best patient outcome. Each approach should be carefully personalized to the patient's condition and concomitant diseases to provide adequate blood pressure and gas exchange and prevent organs and cells from any damage. Cardioprotection, neuroprotection, and ischemia/reperfusion (I/R) protection become important targets in the perioperative period. Thereby, establishment of biochemical blood

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markers detecting organ failures during anesthesia is an important challenge.

Vapor agents are attributed to some protective actions, for example cardioprotective effects of sevoflurane in patients undergoing cardiac surgery, resulting in lower risk of myocardial infarction, reflected by decreasing troponin level, as well as shorter patient hospitalization and reduced 1-year mortality [1–3]. Benefits of vapor anesthetic application to non-cardiac surgery patients are contested as some authors find benefits [4] contrary to others [5]. Similarly, studies in animal models using intravenous anesthetic propofol show some protective effects. At clinically relevant concentrations, propofol can protect immature rabbit hearts from I/R injury [6], augment mitochondrial antioxidant capability in dose-dependent manner during ischemia and reperfusion [7], and reduce reactive oxygen species (ROS) production [8]. Recently, it was shown that propofol was shown to affect expression of matrix metalloproteinases (MMPs) and increase proteolytic activity of MMP-2 and MMP-9 in normoxic rat cardiac fibroblasts [9].

MMPs are a zinc-dependent family of proteolytic enzymes that play a role in extracellular matrix (ECM) remodeling, including morphogenesis, angiogenesis, inflammatory state, tissue repair, and wound healing [10]. In some conditions, MMPs could be released in excess by smooth muscle, platelets, and epithelial or inflammatory cells, resulting in atherosclerotic plaque formation and rupture causing limb or heart ischemia and even myocardial infarction [11]. It is important to note that only the active forms of MMPs play a biological role in cell-to-cell and cell-to-ECM interaction and further remodeling processes [12]. Inactive pro-MMPs are processed into active forms by the influence of cytokines, growth factors, ROS, NO, or activated by proteolytic blood enzymes (thrombin, plasmin, chymase), tissue plasminogen activator, or even hyperglycemia [11]. Homeostasis of all these factors is disturbed during surgical procedures under general anesthesia. These medical processes could be regarded as some kind of controlled trauma, influenced also by the above-mentioned anesthetic agents, which could modulate MMP expression or activity.

High MMP-2 and MMP-9 expression and secretion increase migration ability and invasiveness of breast cancer cell lines [13], as well as enhance cardiac rat fibroblasts cell migration, and invasion [9]. Recently, MMP-9 overexpression was shown to be an important factor in the progression of breast cancer [14], as well as melanoma [15]. Propofol effects on MMP-2 and MMP-9 activities remain unclear; some find its role suppressive [13,16], others opposite [9].

Until now, studies regarding this issue have only been conducted in animals or cell lines, in addition to a single report evaluating MMP-9 concentration in cancer patients [17]. Therefore, there is a need for clinical studies evaluating MMP concentrations and activity after common routine anesthesia during and after surgical procedures. The main goal of our study was to evaluate MMP-2 and MMP-9 concentration and activity, as well as tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) and TIMP-2 concentrations after 2 types of general anesthesia: one based on volatile anesthetic sevoflurane and the second based on intravenous anesthetic propofol during typical non-cancer, non-vascular surgical procedures.

Material and methods

Ethical approval for this prospective observational study (Ethical Committee № KE-0254/142/2011) was provided by the Ethical Committee of Medical University of Lublin, Poland.

After obtaining written consent, 39 patients were included in the analysis. Inclusion criteria were as follows: age above 18 years, assessment with American Society of Anesthesiologists Classification (ASA class) as ASA I and II, elective vertebral column surgery. Exclusion criteria were as follows: assessment as ASA III or more, rheumatoid arthritis, chronic obstructive pulmonary disease, atherosclerosis, diabetes, neurological, immunological and vascular diseases, cancer patients. Fig. 1 shows a detailed chart of the patients flow.

One hour before surgery, patients received diazepam (Relanium) 0.15 mg kg^{-1}, as premedication. During anesthesia, standard monitoring was applied, covering: systolic blood pressure (SAP), diastolic blood pressure (DAP), mean arterial pressure (MAP), heart rate (HR), and pulse oximetry (SpO_2). Moreover, an intra-vein cannula was placed, and the infusion of multi-electrolytic fluid (Plasmalyte) 5-10 ml kg^{-1} h^{-1} was started. Then, patients were randomly divided (simple 1:1 randomization using opaque envelopes) into two groups: group P, with total intravenous anesthesia (TIVA) with propofol (Diprivan) and group S, with volatile induction and maintenance of anesthesia (VIMA) technique applied with sevoflurane (Sevorane).

Anesthetic management in group P included: after pre-oxygenation, atropine (Atropinum Sulfuricum) 0.5 mg and fentanyl (Fentanyl WZF) 3 μg kg^{-1} were given, and the induction of anesthesia with propofol 2–2.5 mg kg^{-1} was started; then, cis-atracurium (Nimbex) 0.1 mg kg^{-1} was administered for neuromuscular blockade and appropriate face mask ventilation intubation was established. Maintenance of anesthesia with propofol (Diprivan) 2–8 mg kg^{-1} h^{-1} infusion (Diprivan) was continued. Additional doses of fentanyl (1.5 μg kg^{-1}) and cisatracurium (0.03 mg kg^{-1}) were added if needed. At the end of surgery, ketoprofen (Ketonal) 100 mg was administered for pain control. Patients were also ventilated with O_2 and an air mixture (35% O_2). Furthermore, end tidal CO_2 was monitored due to normocapnia maintenance (4.0–5.3 kPa). Finally, the neuromuscular blockade was reversed with neostigmine (Polstigminum) 0.04 mg kg^{-1} and with atropine 0.01 mg kg^{-1}.

In group S, after pre-oxygenation, atropine 0.5 mg and fentanyl 3 μg kg^{-1} were given, and induction of anesthesia was started with vital capacity rapid inhalation induction using 4.0% sevoflurane. Then cisatracurium 0.1 mg kg^{-1} was administered for neuromuscular blockade, and after appropriate face mask ventilation, intubation was established. Inhaled sevoflurane 0.8–3.0% for maintenance of anesthesia was applied. Additional doses of fentanyl (1.5 μg kg^{-1}) and cisatracurium (0.03 mg kg^{-1}) were added if needed. At the end of surgery, ketoprofen 100 mg was administered for pain control. Patients were also ventilated with O_2 and air mixture (35% O_2). Furthermore, end tidal CO_2 was monitored due to normocapnia maintenance (4.0–5.3 kPa). Finally, the neuromuscular blockade was reversed with neostigmine 0.04 mg kg^{-1} and with atropine 0.01 mg kg^{-1}.

The typical postoperative pain regimen comprised of ketoprofen and tramadol administration for each patient. Postoperative pain intensity was evaluated every 4 h during the first postoperative day using a numeric analogue scale (NAS), scored 0-10. If a patient scored more than 3 in NAS, an additional meperidine dose was administered (apart from ketoprofen and tramadol).

Just before anesthetic management (prior to premedication) and 24 h after surgery, venous blood was collected into 10-ml blood collection tubes with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Blood samples were centrifuged at 2500 x g for 10 min. Plasma was removed immediately, aliquoted, and stored at −80 °C. After thawing, samples were re-centrifuged at 1000 x g for 15 min to remove any cell debris. Then, enzyme-linked immunosorbent assay (ELISA) was used to measure MMP-2, MMP-9, TIMP-1, and TIMP-2 concentrations. Additionally, MMP-2 and MMP-9 activities by means of gelatin zymography were assessed. Patient characteristics are presented in the Table 1. Twenty patients were anesthetized

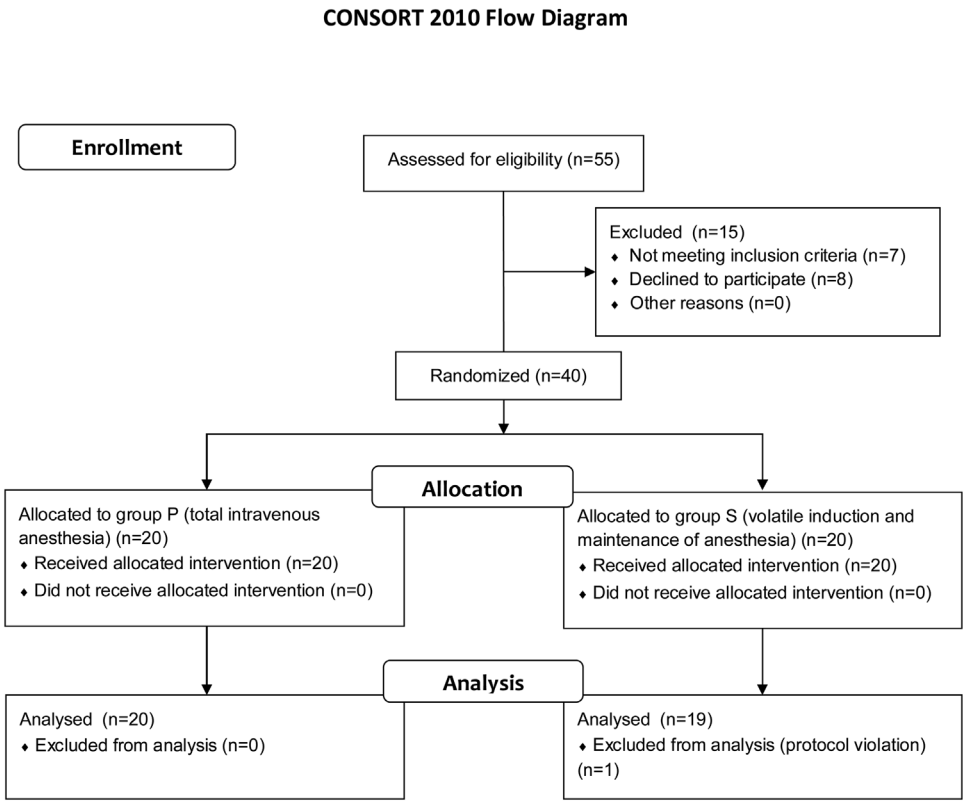

Fig. 1. Chart of the patients' flow.

Table 1
Patient characteristics.

	GROUP P (n = 20)	GROUP S (n = 19)	*p* value
Age (years)[a]	51 ± 11	48 ± 12	0.516
Sex			
Women[b]	13 (65)	10 (53)	0.433
Men[b]	7 (35)	9 (47)	
Weight (kg)[a]	82 ± 17	77 ± 16	0.372
Height (cm)[a]	168 ± 10	171 ± 8	0.365
BMI (kg m^{-2})[a]	29 ± 5	26 ± 5	0.103
Surgery time (min)[a]	97 ± 53	91 ± 43	0.955
Anesthesia time (min)[a]	135 ± 56	128 ± 44	0.922
Meperidine TAP[b]	13 (65)	13 (68)	0.844

BMI – body mass index; TAP - treatment after procedure.
[a] Values are presented as mean ± standard deviation.
[b] Values are presented as numbers, and, in parentheses, as percentages.

with total intravenous anesthesia with propofol (group P) and 19 with volatile induction and maintenance of anesthesia technique using sevoflurane (group S).

ELISA assay

ELISA assays of MMP-2, MMP-9, TIMP-1, and TIMP2 were performed according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Gelatin zymography

MMP-2 and MMP-9 activities were determined by gelatin zymography according to the method described previously [18,19]. Briefly, the samples in a volume of 15 μl consisting of plasma and sample buffer with 10% sodium dodecyl sulfate (SDS) in a ratio of 4:1 were separated in 10% polyacrylamide gels containing 0.05% gelatin type A from porcine skin (Sigma-Aldrich, St. Louis, MO, USA). Following electrophoresis, two 30-min washes were done in washing buffer (50 mM Tris–HCl, pH 7.2 10 mM $CaCl_2$, 0.02% NaN_3, and 2.5% Triton X-100). Further incubation with the above-mentioned buffer containing 1% Triton X-100 (18 h at 37 °C) results in re-activation of gelatinases in experimental conditions based on gelatin degradation. In gel, incorporated gelatin is degraded by re-activated gelatinases (MMP-2/MMP-9) at the place of their molecular weight-dependent electrophoretic position (66 kDa and 84 kDa respectively), resulting in the appearance of white bands after staining. Gels were stained in 0.1% Coomassie Brillant Blue R-250 (Bio-Rad Laboratories, United Kingdom) in 30% ethanol and 10% acetic acid and destained in 30% ethanol and 10% acetic acid. The activities of MMP-2 and MMP-9 were detected as cleared, unstained regions on the blue background. Enzymes were identified by comparing their migration to and MMP-2 and MMP-9 standards (R&D Systems, Minneapolis, MN, USA) and a molecular mass standard (ThermoFisher Scientific, USA). For objective quantification of bands, we used densitometry with the ImageJ software and peak area for each band was determined (greater area - greater activity), as described previously [19].

Statistical analysis

Statistical analysis was performed using STATISTICA software (StatSoft Polska Sp. z o.o., Kraków, Poland), with a significance level of <0.05. Primary endpoint was determined as MMP-9 concentration 24 h after anesthesia. Sample size was calculated using a STATISTICA Power Analysis module and determined at a minimum level of 17 subjects per group. Assumptions for primary endpoint were as follows: baseline MMP-9 concentration (mean 1 ± standard deviation): 36 ± 8, MMP-9 concentration 24 h after procedure (mean 2 ± standard deviation): 45 ± 9.9 (as we assumed 25% increase), alpha = 0.05, power goal 0.8. Patient's characteristic

Table 2
MMP-2, MMP-9, TIMP-1, and TIMP-2 plasma concentrations before and 24 h after surgery.

	BASELINE MED (IQR)	24 h AFTER SURGERY MED (IQR)	% OF CHANGE	*p*	statistical test
TOTAL INTRAVENOUS ANASTHESIA (GROUP P, n = 20)					
MMP-2 (ng/ml)	1.2 (0.8-1.2)	0.9 (0.8-1.0)	−25	0.044	Wilcoxon
MMP-9 (ng/ml)	5.8 (4.1-9.1)	8.6 (5.9-15.0)	+48	0.011	Wilcoxon
TIMP-1 (ng/ml)	1.7 (1.1-2.0)	0.8 (0.6-1.4)	−47	0.002	t-test
TIMP-2 (ng/ml)	1.4 (1.1-2.2)	1.1 (0.6-1.4)	−21	0.097	t-test
VOILTAILE INDUCTION AND MAINTENANCE OF ANESTHESIA (GROUP S, n = 19)					
MMP-2 (ng/ml)	1.0 (0.8-1.2)	0.8 (0.6-1.0)	−20	0.027	Wilcoxon
MMP-9 (ng/ml)	5.1 (3.7-8.0)	10.7 (6.0-14.1)	+110	0.001	Wilcoxon
TIMP-1 (ng/ml)	1.9 (1.4-2.6)	0.9 (0.6-1.4)	−47	0.000	t-test
TIMP-2 (ng/ml)	1.4 (1.2-1.7)	1.3 (0.6-1.7)	−7	0.172	t-test

MED - median; IQR - interquartile range.

data were presented as mean and standard deviation, and other data was presented as median and quartile range. All data was checked due to normality distribution (Shapiro-Wilk's W test) and variance equality when appropriate. Meeting these assumptions, data were tested with parametric *t*-test (two-tailed) for dependent or independent samples properly. Otherwise were tested with non-parametric Wilcoxon test for dependent or Mann-Whitney U test for independent samples. Sex differences were tested with the non-parametric chi-squared test. For correlations, Spearman's rank correlation coefficients were calculated.

Results

No statistical differences were found between groups (S or P) concerning patients' characteristics or anesthesia and surgery duration (Table 1). Twenty-four hours after the surgical procedure, a significant decrease in the plasma MMP-2 concentration and an increase in MMP-9 concentration were detected in both patient groups: P and S. Analyzing TIMPs plasma concentrations, we observed significant decreases in TIMP-1 concentration 24 h after surgery without differences between the P and S groups, whereas TIMP-2 concentration remain unchanged 24 h after procedure among both groups (Table 2). Data analysis in regard to anesthesia (propofol or sevoflurane) impact on MMP-2 and MMP-9 concentrations revealed no differences between groups (Table 3). Although the MMP-9 concentration was higher in the sevoflurane group, it was not statistically significant. The same result concerns TIMP-1 and TIMP-2 concentrations, no differences between group P and S 24 h after procedure (Table 3) were observed.

Our study demonstrates that surgical procedures affected not only concentrations, but also plasma MMP-2 and MMP-9 activities, analyzed by means of gelatin zymography (Fig. 2). This method revealed that both forms of MMP-2 enzyme (72 kDa non-active proenzyme and 66 kDa active enzyme), as well as MMP-9 enzyme (92 kDa non-active proenzyme and 84 kDa active enzyme) are present in plasma. In experimental conditions, all forms (proenzyme and "working" enzyme) were re-activated, regardless of the type of anesthesia used. However, we detected very low activity of active forms for both enzymes (lower bands), suggesting that, in reality, MMP-2 and MMP-9 are weakly activated by both types of anesthesia.

Densitometric analyses demonstrated that the average gelatinolytic activity were higher 24 h after procedure for both MMP-9 ($p = 0.000$) and MMP-2 ($p = 0.000$), but there were no differences between groups (Fig. 3). Detailed densitometric data, presented as peak area for each band, are attached as supplementary materials (Suppl. 1).

Due to lack of differences in MMP concentrations and activity in regard to anesthetic used, we analyzed data in regard to additional pharmacotherapy on MMP concentrations and activity. We found that patients with higher pain sensation expressed as NAS > 3 and requiring additional pain killer meperidine revealed lower MMP-9 activity 24 h after surgery (detected by zymography), meperidine use correlated with lower MMP-9 activity (R = - 0.430, $p = 0.006$) (Table 4).

Table 3
MMP-2, MMP-9, TIMP-1, and TIMP-2 plasma concentrations before and 24 h after anesthesia with propofol (group P) and sevoflurane (group S).

	PROPOFOL (Group P, n = 20) MED (IQR)	SEVOFLURANE (Group S, n = 19) MED (IQR)	*p*	statistical test
BASELINE				
MMP-2 (ng/ml)	1.2 (0.8-1.2)	1.0 (0.8-1.2)	0.603	Mann-Whitney U
MMP-9 (ng/ml)	5.8 (4.1-9.1)	5.1 (3.7-8.0)	0.500	Mann-Whitney U
TIMP-1 (ng/ml)	1.7 (1.1-2.0)	1.9 (1.4-2.6)	0.236	t-test
TIMP-2(ng/ml)	1.4 (1.1-2.2)	1.4 (1.2-1.7)	0.937	t-test
24 h AFTER PROCEDURE				
MMP-2 (ng/ml)	0.9 (0.8-1.0)	0.8 (0.6-1.0)	0.457	Mann-Whitney U
MMP-9 (ng/ml)	8.6 (5.9-15.0)	10.7 (6.0-14.1)	0.757	Mann-Whitney U
TIMP-1 (ng/ml)	0.8 (0.6-1.4)	0.9 (0.6-1.4)	0.736	t-test
TIMP-2 (ng/ml)	1.1 (0.6-1.4)	1.3 (0.6-1.7)	0.635	t-test

MED - median; IQR - interquartile range.

Discussion

General anesthesia is applied by two approaches based on propofol infusion or inhaled agents, such as sevoflurane. Each of them has advantages, and thus the discussion regarding which is superior remains open. Propofol was found to have some cardiac protective effects against I/R injury by decreasing ROS generation [7] and is supposed to diminish angiotensin II induced cardiomyocyte hypertrophy, which plays a role in the cardiac remodeling process after I/R injury [20]. Nevertheless, clinical implications of propofol protective effects remain unclear and its impact on morbidity or mortality is still vague.

Sevoflurane is a second anesthetic often used in clinical application for general anesthesia. Sevoflurane use is encouraged due to its potential organ-protective effect. De Conno [21] found increased inflammatory mediators and more adverse events in propofol anesthetized patients versus inhaled anesthesia with sevoflurane for thoracic surgery. Sevoflurane is also considered to be cardioprotective for cardiac surgery patients with I/R events [1–3]. It was reported that sevoflurane could influence potassium adenosine triphosphate (K_{ATP}) channels, ROS production, mitochondrial transition pore (mPTP) permeability, and also on cytokines and extracellular-signal kinases (ERK) activity [22].

Potential influence of propofol on MMPs expressions and activation was demonstrated previously *in vitro*. Qing [13] revealed that propofol reduced MMP-2 and MMP-9 expression

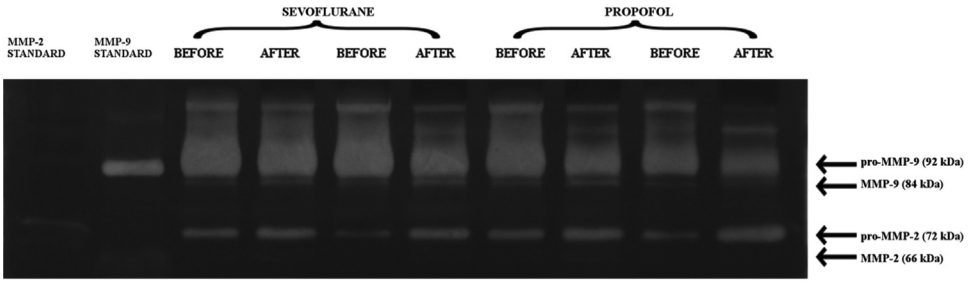

Fig. 2. Gelatin zymography showing MMP-2 and MMP-9 activity before and 24 h after two types of anesthesia.
On the presented zymogram positions of pro-enzyme (upper bands) and its active forms (lower bands) are indicated by arrows according to the positions of MMP-2 and MMP-9 standard markers. Description of the samples is indicated at the top of the gel.

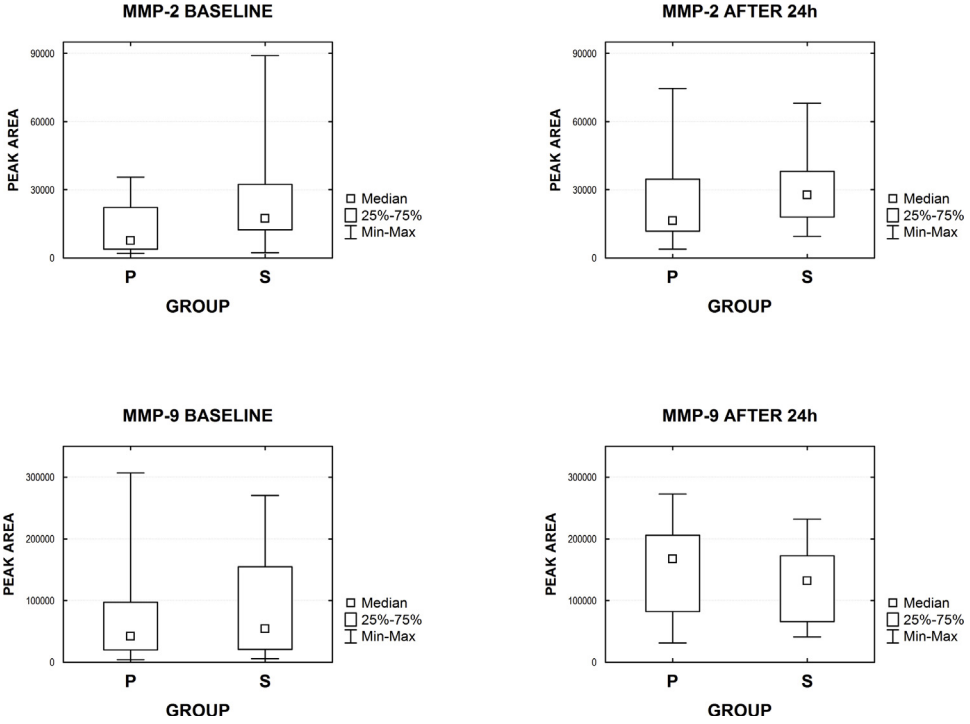

Fig. 3. The differences between MMP activity before and 24 h after two types of anesthesia.

Table 4
MMP-2, MMP-9, TIMP-1, and TIMP-2 plasma concentrations and MMP-2 and MMP-9 activity (gelatin zymography) in relation to meperidine treatment.

	NAS $\leq$ 3 WITHOUT MEPERIDINE MED (IQR)	NAS > 3 WITH MEPERIDINE MED (IQR)	*p*	statistical test	correlation
	BASELINE				
MMP-2 (ng/ml)	1.2 (1.0-1.2)	1.0 (0.7-1.2)	0.251	Mann-Whitney U	R=-0.186; $p = 0.257$
MMP-9 (ng/ml)	5.5 (3.7-8.0)	5.8 (3.8-9.0)	0.551	Mann-Whitney U	R = 0.097; $p = 0.558$
MMP-2 activity (peak area)	[12.4 (5.1-19.3)] x 10^3	13.4 (5.0-23.5)] x 10^3	0.789	Mann-Whitney U	R = 0.043; $p = 0.793$
MMP-9 activity (peak area)	[42.9 (23.4-126.9)] x 10^3	[55.2 (19.7-89.8)] x 10^3	0.905	Mann-Whitney U	R=-0.019; $p = 0.907$
TIMP-1 (ng/ml)	1.8 (0.9-1.9)	1.8 (1.4-2.4)	0.202	t-test	R = 0.176; $p = 0.283$
TIMP-2 (ng/ml)	1.7 (1.2-2.4)	1.4 (1.2-1.7)	0.223	t-test	R=-0.218; p=0.183
	24 h AFTER PROCEDURE				
MMP-2 (ng/ml)	0.9 (0.7-0.9)	0.9 (0.7-1.0)	0.666	Mann-Whitney U	R = 0.070; $p = 0.672$
MMP-9 (ng/ml)	14.1 (6.5-15.1)	9.2 (6.0-13.1)	0.245	Mann-Whitney U	R=-0.188; $p = 0.251$
MMP-2 activity (peak area)	[23.5 (15.7-37.6)] x 10^3	[24.4(12.7-35.2)] x 10^3	0.858	Mann-Whitney U	R=-0.029; $p = 0.861$
MMP-9 activity (peak area)	[213.4 (175.9-222.4)] x 10^3	131.6 (77.6-166.1)] x 10^3	0.008	Mann-Whitney U	R=-0.430, $p = 0.006$
TIMP-1 (ng/ml)	0.8 (0.6-1.2)	0.9 (0.6-1.5)	0.432	t-test	R = 0.109; $p = 0.509$
TIMP-2 (ng/ml)	1.1 (0.6-1.4)	1.2 (0.5-1.5)	0.725	t-test	R=-0.339; $p = 0.838$

NAS - numeric analogue scale; MED - median; IQR - interquartile range.

and secretion on breast cancer cell lines as well as reduced migration ability and invasiveness of breast cancer cells. Similarly, Xu [16] showed that treatment with propofol significantly inhibited the expression of MMP-9 protein in a dose- and time-dependent manner in esophageal squamous cell carcinoma cells. In contrast, Jun et al. [9] noticed that propofol increased proteolytic activity of MMP-2 and MMP-9 and enhanced cell migration and invasion of cardiac rat fibroblasts. These discrepancies could be related to the cell type used for these studies, especially that cancer cells have different metabolism than normal cells. Furthermore, *in vitro* studies or in animal models do not always reflect findings observed in clinical studies.

It appears desirable to decrease MMP-2 and MMP-9 concentrations and activity after oncological surgery, especially after breast cancer and melanoma surgery. It was discovered that MMP-2 play a role in breast cancer progression [23], as well as MMP-9 play an important role in poor prognosis in tumor cells metastasis [24]. Meta-analysis concerning the impact of MMP-2 on progression and clinicopathology of breast cancer patient showed that positive MMP-2 expression could be a predictive factor for poor prognosis in breast cancer patients [25]. Meta-analysis of MMP-9 expression as a prognostic value in breast cancer patients revealed similar findings [26]. The results of the above-mentioned studies suggest that positive MMP-9 expression correlate with relapse and a worse survival in breast cancer patients. Latest meta-analysis [27] confirms that MMPs overexpression, especially MMP-2 and MMP-9, could indicate a higher risk of poor prognosis in breast cancer. Very similar findings were reported with metastatic melanoma patients [15]. The authors concluded that MMP-1, MMP-9 and MMP-13 played a role in the spreading of metastatic melanoma and that MMP-9 might have clinical value as a risk factor for melanoma progression. All above-mentioned clinical failures might be an effect of cancer cell lines migration and invasiveness caused by high MMP-2 and 9 expression and secretion [13]. To summarize, it would be beneficial for an anesthetic agent to trigger depression of MMP-2 and MMP-9 expression to support oncological treatment.

There are few reports describing differences in MMP concentrations in clinical settings involving surgery and anesthesia. Increased MMP-9 concentration was observed 120 min after cardiac surgery [28] and after extracorporeal circulation (ECC) [29,30]. Volatile anesthetics (including sevoflurane) have been shown to decrease MMP-9 concentration after ECC [31] and after breast cancer surgery in patients anesthetized with combined general/regional anesthesia (propofol/paravertebral blockade) versus sevoflurane/opioid anesthesia [17], which is in conflict with the results obtained in our current study. However, it is not possible to distinguish whether propofol or paravertebral blockade is responsible for decreasing MMP-9 concentration. In contrast, increased activity of matrix metalloproteinase 9 was observed in sevoflurane-treated animals in comparison to propofol anesthetized pigs in the cardiopulmonary resuscitation model [32]. Sevoflurane pretreatment increased the concentrations of MMP-2 and MMP-9 in an animal ischemia model [33], which is in line with our results.

This question of functional significance of MMP amount and activity during and after surgical procedures and anesthesia remains open. Trauma, hemodynamic instability, oxidative stress, and inflammation could influence MMPs expression and activation [11]. Some of these factors occurs during typical surgical procedure, which is a type of control trauma for a patient and lead to MMP activation to start tissue remodeling, angiogenesis and wound healing [34]. Our research revealed that MMP-2 concentration decreased, while activity increased. This might be due to active and non-active forms present in the plasma and surgery, as traumatic process might activate MMP-2 through the factors mentioned above. On the other hand, it could parallelly influence atherosclerotic plaque formation, rebuilding [11], or even its instability and rupture [35]. Because only active form of MMPs play a biological role in cell-to-cell and cell-to-ECM interaction and further remodeling processes [12], low amounts of MMP's active forms in the sera of anesthetized patients observed in our study suggest that both types of anesthesia are rather safe in these aspects.

Using sevoflurane seems to be more advantageous due to its anti-inflammatory properties and potentially plaque-stabilizing effect [36]. Sevoflurane was also more active than propofol in reducing inflammatory mediators' concentrations (tumor necrosis factor α, interleukin 6 and 8 and monocyte chemoattractant protein 1) in bronchoalveolar lavage fluid [21]. To support these findings with clinical settings, Von der Linden [37] found no cardiovascular complications in patients anesthetized with sevoflurane *vs.* 4 complications and 3 depths with propofol anesthesia technique. On the other hand, de Heart [38] showed no difference in cardiac events between volatile *vs.* non-volatile anesthetized patients.

An additional interesting finding of our study was that supplementary pharmacotherapy may influence MMP concentrations, as demonstrated for meperidine. Decreased MMP-9 activity might be beneficial for oncological patients, especially breast cancer and melanoma patients, although these findings are limited by the small number of patients enrolled in our study. These uncertainties require further study on a larger population to elicit any additional interactions between drugs and MMP concentrations or activity.

A limitation of our study is the use of ketoprofen as a pain killer for patients during the postoperative period. This is a non-steroidal antirheumatic and a strong anti-inflammatory substance, which could affect MMP expression. Ketoprofen decrease MMP-2 expression, tumor growth, and pulmonary metastatic incidence in nude mice with osteosarcoma [39]. Further evaluation is needed to determine whether this might be beneficial during oncological treatment.

In conclusion, neither sevoflurane nor propofol used as anesthetics modulate MMP-2 and MMP-9 concentrations or activities during non-oncological, non-vascular elective surgery. Meperidine use as a pain killer seems to decrease MMP-9 activity.

Conflicts of interest

None declared.

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