



SCIENTIFIC ARTICLE

Comparison of effects on the oxidant/antioxidant system of sevoflurane, desflurane and propofol infusion during general anesthesia



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KEYWORDS

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Abstract

Background and objectives: Desflurane and sevoflurane are frequently used for maintenance of anesthesia and studies have shown that these anesthetics cause a variety of changes to the oxidative stress and antioxidative defense mechanisms. This study aims to compare the effects of sevoflurane, desflurane and propofol infusion anesthesia on the oxidant and antioxidant systems of patients undergoing laparoscopic cholecystectomy.

Methods: 45 patients between 18 and 50 years with planned laparoscopic cholecystectomy under general anesthetic were included in the study. Patients were divided into three groups on the way to surgery: propofol (group P n: 15), sevoflurane (group S n: 15) and desflurane (group D n: 15). All groups were given hypnotic 2 mg/kg propofol IV, 1 mcg/kg fentanyl IV and 0.1 mg/kg vecuronium IV for induction. For maintenance of anesthesia group S were ventilated with 2% sevoflurane, group D cases were given 6% desflurane and group P were given propofol infusions of 12 mg/kg/h for the first 10 min, 9 mg/kg/h for the second 10 min and 6 mg/kg/h after that. Before induction and after the operation venous blood samples were taken to evaluate the levels of glutathione peroxidase, total oxidants and antioxidants.

Results and conclusions: The 45 patients included in the study were 22 male and 23 female patients. The demographic characteristics of the groups were similar. In the postoperative period we observed that while sevoflurane and propofol increased antioxidants by a statistically significant level, desflurane increased the total oxidants level by a significant amount compared to levels before the operation.

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PALAVRAS-CHAVE

Oxidante;
Antioxidante;
Propofol;
Anestesia geral

Comparação dos efeitos da perfusão de sevoflurano, desflurano e propofol sobre o sistema oxidante/antioxidante durante anestesia geral**Resumo**

Justificativa e objetivos: Desflurano e sevoflurano são usados com frequência para a manutenção da anestesia, e estudos mostraram que esses anestésicos causam alterações variadas nos mecanismos de defesa antioxidante contra o estresse oxidativo. Este estudo teve como objetivo comparar os efeitos de anestésias com perfusão de sevoflurano, desflurano e propofol sobre os sistemas oxidante/antioxidante de pacientes submetidos à colecistectomia laparoscópica.

Métodos: Foram incluídos no estudo 45 pacientes entre 18 e 50 anos, agendados para colecistectomia laparoscópica sob anestesia geral foram incluídos no estudo. Os pacientes foram divididos em três grupos para receberem propofol (Grupo P, *n*: 15), sevoflurano (Grupo S, *n*: 15) e desflurano (Grupo D, *n*: 15). Todos os grupos receberam 2 mg/kg de propofol IV, 1 mcg/kg de fentanil IV e 0,1 mg/kg de vecurônio IV para indução. Para manutenção da anestesia, o Grupo S recebeu ventilação com sevoflurano a 2%, o Grupo D recebeu desflurano a 6% e o Grupo P recebeu propofol em perfusões de 12 mg/kg/h nos primeiros 10 min, 9 mg/kg/h nos 10 min seguintes e 6 mg/kg/h subsequentemente. Antes da indução e depois da cirurgia, amostras de sangue venoso foram colhidas para avaliar os níveis de glutatona peroxidase e o total de oxidantes e antioxidantes.

Resultados e conclusões: Dos 45 pacientes incluídos no estudo, 22 eram do sexo masculino e 23 do sexo feminino. As características demográficas dos grupos eram semelhantes. No período pós-operatório, observamos que enquanto sevoflurano e propofol aumentaram os antioxidantes a um nível de significância estatística, desflurano aumentou o nível total de oxidantes em quantidade significativa, em comparação com os níveis pré-operação.

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Introduction

Free oxygen radicals, on the one hand while they oxidize biological molecules such as the body's building blocks of protein, lipid and DNA, on the other hand they can work against this oxidation as part of the body's natural antioxidant defense system. This situation is balanced under normal physiological conditions. However in a situation of meeting any stress, as a result of increasing antioxidant consumption or free radical creation, many oxidative stresses increase.^{1,2} Many antioxidant molecules are found in the blood to prevent or inhibit the harmful effects of free oxygen radicals. Measurement of total antioxidant and oxidant levels in plasma can be used to determine the oxidative stress reaction of the organism.^{3,4} Desflurane and sevoflurane are frequently used for maintenance of anesthesia and studies have shown that these anesthetics cause a variety of changes to the oxidative stress and antioxidative defense mechanisms.⁵ The chemical structure of propofol is similar to some free radical consumers such as endogenous vitamin E and butylated hydroxytoluene.⁶⁻⁸

In this study we aimed to compare the effects of sevoflurane, desflurane and propofol infusion anesthesia on the oxidant and antioxidant systems of patients undergoing laparoscopic cholecystectomy.

Methods

After permission was given by 2009/12 no. decision of Duzce University Medical Faculty Clinical Study Ethics Committee

and informed patient consent was obtained, 45 patients ASA I–II between 18 and 50 years with planned laparoscopic cholecystectomy under general anesthetic were included in the study. Patients were divided into three groups on the way to surgery: TIVA (group P *n*: 15), sevoflurane (group S *n*: 15) and desflurane (group D *n*: 15). Patients with endocrine dysfunction, blood transfusion in the previous 2 weeks, signs of infection and inflammation, history of preoperative medication use, anemia, and hemorrhage requiring transfusion during the operation were excluded from the study. After fasting for 8 h all patients in the study were given 1 mg midazolam IV for premedication 30 min before the operation. Patients were taken to the operating room. Routine monitoring with electrocardiogram (ECG), peripheral oxygen saturation (SPO₂), and non-invasive blood pressure were done. Later before the operation venous blood was taken to evaluate total oxidant status, total antioxidant status and glutathione peroxidase levels.

All groups were given hypnotic 2 mg/kg propofol IV, 1 mcg/kg fentanyl IV and 0.1 mg/kg vecuronium IV for induction. During anesthesia induction cases were oxygenized with 100% O₂ at 6 L/min flow rate. After 3 min of controlled ventilation, using an appropriate intubation tube for age and weight, orotracheal intubation was completed. For maintenance of anesthesia, group S were ventilated with 2% sevoflurane, 50% air and 50% O₂ mix at 6 L/min flow. Group D cases were given 6% desflurane, 50% air and 50% O₂ mix at 6 L/min flow. Group P were given propofol infusions of 12 mg/kg/h for the first 10 min, 9 mg/kg/h for the second 10 min and 6 mg/kg/h after that. Patients were ventilated with 50% air and 50% O₂ mix at 6 L/min flow. In all groups

after tidal volume was determined to be 6–8 mg/kg and respiration rate was 12, ventilation was begun with an Avance S/5 anesthesia device. Ten minutes before the expected end of surgery propofol infusion was ended. When the last skin suture was being made inhalation agents were shut off. Manual ventilation with 100% O₂ was undertaken. In this period all parametric readings were taken. After spontaneous respiration began, neuromuscular antagonization was completed with 0.01 mg/kg atropine and 0.03 mg/kg neostigmine. After extubation the patient was taken to recovery. Later venous blood was taken to evaluate total oxidant status, total antioxidant status and glutathione peroxidase levels after the operation.

Biochemical analysis

Blood samples taken in anticoagulant free vacuum gel tubes were centrifuged at 2000 × *g* for 15 min. The serum was portioned into clean tubes. For glutathione studies the serum separated in the tube was deproteinized. For the deproteinization procedure 5 g metaphosphoric acid was dissolved in 50 mL distilled water and 200 μL samples were mixed with 200 μL metaphosphoric acid solution and vortexed. They were rested at room temperature for 5 min and were centrifuged at 2000 × *g* for 4 min. The supernatant was carefully separated to a different tube. This supernatant to measure glutathione and the serum portions designated to measure other parameters were stored at –80 °C until measurements could be made. Glutathione was measured based on enzymatic measurements using Cayman (Cayman Inc., Ann Arbor, MI, USA) commercial kits, which use 5-thio-2-nitrobenzoic acid (TNB) to react with the DTNB (5,5'-dithio-bis-2-nitrobenzoic acid) of the sulfhydryl group in GSH glutathione reductase forming a yellow color. On the day of measurement, samples were thawed and mixed, then prepared 4 M triethanolamine solution of 10 μL was added to 200 μL samples and vortexed. After samples were diluted at 1:2 (v/v) ratio with MES buffer, standards were prepared according to the prospectus. They were added to 50 μm wells, and the plate was covered with the lid from within the kit. At this stage, according to the prospectus an assay cocktail of MES buffer (11.25 mL), GSH co-factor mixture (0.45 mL), GSH enzyme mixture (2.1 mL), distilled water (2.3 mL), and GSH DTNB (0.45 mL) was prepared and 150 μL freshly-prepared assay cocktail was added to all wells, the plate was covered and incubated on an orbital mixer in the dark for 25 min. In the 25th minute measurement was taken using a Bio-Rad 680 Microplate reader at 414 nm. Total antioxidant measurements used a Cayman (Cayman Inc., Ann Arbor, MI, USA) commercial kit based on oxidation inhibition of ABTS*+ by the metmyoglobin in ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]). On the day of measurement samples were thawed and mixed, then for antioxidant measurements the samples were diluted with assay buffer at 1:19 (v/v) and standards were prepared according to the prospectus. After 10 μL trolox standard or 10 μL sample was added to all wells 10 μL metmyoglobin and 150 μL chromogen solution were added and to begin the reaction immediately 40 μL 441 μM antioxidant assay hydrogen peroxide was added. The plate was covered and incubated on a mixer at room temperature for 5 min. Measurement was made using a Bio-Rad 680 Microplate reader

at 405 nm. Total oxidant capacity measurements used a Rel Assay kit (Rel Assay Diagnostics, Mega Tip San and Tic Ltd Sti, Turkey) developed by Erel.⁹ This method is based on oxidants in the sample turning a ferrous iron chelator into ferric ions, which in an acid environment form a color complex with chromogen and this complex is then measured spectrophotometrically. Stabilized stock standard solution (SSSS) was diluted 40,000 times by deionized water then 150 μL or the sample was mixed with 1000 μL reactive I. First absorbance was measured using a spectrophotometer at 530 nm wavelength. Fifty μL prochromogen solution was added, mixed and incubated at room temperature for 10 min. Second absorbance was measured at 530 nm.

For calculations the following formulae were used:

$$\text{TOS} = \left(\frac{\Delta \text{Absorbance sample}}{\Delta \text{Absorbance standard}} \right) \times \text{standard value}$$

$\Delta \text{Absorbance sample} = 2\text{nd absorbance sample} - 1\text{st absorbance sample}$,

$\Delta \text{Absorbance standard} = 2\text{nd absorbance standard} - 1\text{st absorbance standard}$,

Standard value: 20 μmol H₂O₂ equiv./L.

Statistical analysis

Data were analyzed using the SPSS v 11.5 (SPSS, Inc., Chicago, IL, USA) computer program. Distribution of numerical data within the groups was evaluated using the Shapiro–Wilk test. Data with normal distribution are given as mean ± standard deviation (SD); categorical variables are given as frequencies. To determine any differences in average numerical data between the groups the one-way ANOVA was used. To find groups responsible for significant differences multivariate analysis used the Scheffe test. Before and after measurements were analyzed with the paired samples *t* test as they had normal distribution. Categorical variables were compared between the groups with the chi-square test. A *p* value <0.05 was accepted as statistically significant.

Results

Our study comprised a total of 45 patients: 15 were given sevoflurane, 15 were given desflurane and 15 were given propofol infusion. Of the 45 patients 22 were male and 23 were female patients. There was no statistical difference between the patients in the groups in terms of average age, weight, and anesthesia duration (Table 1) (*p* > 0.05). Hemo-

Table 1 Demographic characteristics of patients.

	Group S	Group D	Group P	<i>p</i>
Age (years)	35 ± 8	36 ± 8	34 ± 8	0.84
Heavy (kg)	69 ± 9.3	68 ± 8.8	67 ± 8.6	0.92
Sex (M/F)	9/6	7/8	6/9	
Anesthesia time (min)	106 ± 13	113 ± 18	107 ± 10	0.43

M, male; F, female.

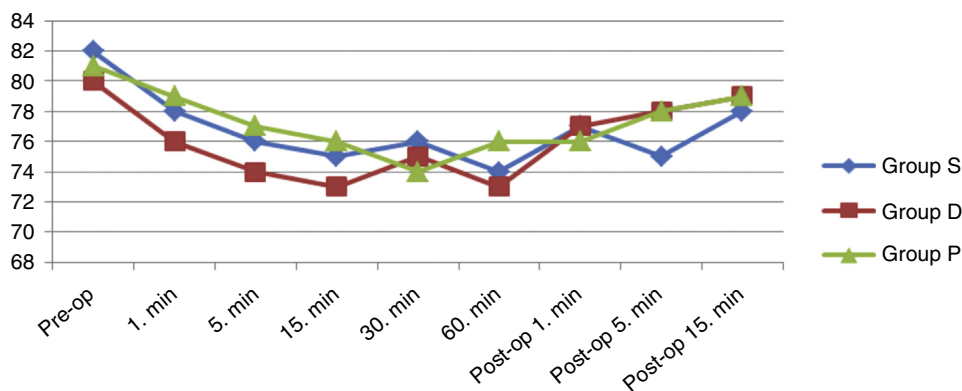


Figure 1 Hemodynamic data from the groups.

Table 2 TAS, TOS and GSH-PX levels of patients.

	PREOP	POSTOP	<i>p</i>
TAS (mmol/L)			
Group S	0.6 ± 0.1	0.8 ± 0.2	0.02 ^a
Group D	0.8 ± 0.2	0.7 ± 0.2	0.24
Group P	0.6 ± 0.1	0.7 ± 0.1	0.04 ^a
TOS (μmol/L)			
Group S	7.1 ± 4.5	10.5 ± 6.4	0.07
Group D	9.1 ± 4.6	12.6 ± 4.1	0.03 ^b
Group P	8.4 ± 6.3	6.4 ± 4.2	0.10
GSH-PX (mmol/L)			
Group S	0.7 ± 0	0.7 ± 0.1	0.42
Group D	0.7 ± 0.1	0.7 ± 0.1	0.89
Group P	0.8 ± 0.1	0.7 ± 0.1	0.79

^a There is statistically significant difference in TAS between the preoperative and postoperative period in groups S and P.

^b There is statistically significant difference in TOS between the preoperative and postoperative period in group D.

dynamic monitoring of patients was completed during the operation. During this monitoring the patients' preoperative, intraoperative and postoperative mean blood pressure (MBP) was recorded (Fig. 1). The MBP values of patients in groups S, D and P were similar. It was observed that sevoflurane and propofol significantly increases the total antioxidant capacity. Furthermore we found that desflurane significantly increases the total oxidant capacity.

The TAS, TOS and glutathione peroxidase levels of patients before and after the operation are shown in Table 2.

Discussion

In this study we reached the conclusion that sevoflurane and propofol have antioxidant properties while desflurane increased oxidative stress.

The aim of general anesthetic applications is to create anesthesia effectively while reducing to minimal levels the conditions that may harm the organism. Anesthetic material appropriate for this aim should be pure and stable chemically, have quick onset and slow end to effect, and not create any unwanted effects on vital functions during and after administration.¹⁰ In fact anesthetic materials and

anesthetic duration used in general anesthesia, together with the stress of surgical trauma, are important factors that disrupt the body's immunological and antioxidant defense systems.¹¹ Laparoscopic surgery exposes the parietal peritoneum and visceral peritoneum to ischemic trauma. Studies have shown that if intraabdominal pressure is held at 15 mmHg during laparoscopy, blood flow to the parietal peritoneum reduces by a significant amount, while at 10 mmHg there is no change. For this reason pneumoperitoneum may potentially cause ischemia and increase free oxygen radicals.¹² General anesthesia disrupts the immunological defense mechanisms and induces an inflammatory reaction in alveolar macrophages. In generalized inflammatory reactions including production of leukocytes, inflammation mediators and free oxygen radicals are released. Membrane damage caused by free radicals during general anesthesia presents as observed lipid peroxidation products.¹³ Studies have shown that a variety of medications used in anesthesia have effects on the oxidant-antioxidant system. However the effect on the antioxidant system of the inhalation anesthetic desflurane has not been fully studied. Knowledge of the interactions of inhalation agent used on patients under oxidative stress carries clinical importance.¹⁴ Baysal et al.¹⁵ examined the total oxidative status and antioxidative status levels of pediatric patients undergoing laparoscopic surgery and concluded that the surgical stress of laparoscopy with inhalation anesthetics increased total oxidant capacity and reduced total antioxidant capacity.

In recent years many studies on the antioxidant system have been completed, these studies found that the antioxidant system has major effects on patient mortality and morbidity.¹⁶ Antioxidant systems normally work as a whole, protecting cells from the toxic effects of oxygen radicals. This maintains the oxidant and antioxidant systems in the organism in a balanced fashion. In situations where this balance is disrupted toward oxidation, inflammatory mediators and free oxygen radicals are produced by leukocytes. These create lipid peroxidation in cell membranes, damaging DNA and causing disease.¹⁷

In our study we observed in the desflurane group total oxidant capacity, an indicator of oxidative stress, was statistically increased, while antioxidant capacity was reduced, though not at statistically significant levels. In the sevoflurane group oxidative stress was increased compared to values before the operation, though not significantly,

while antioxidant capacity was statistically significantly increased. Dikmen et al.¹⁸ looked at the effect of sevoflurane on the enzymatic antioxidant defense system in the livers of rats and showed that sevoflurane produced an increase in lipid peroxidation before any insufficiency in the enzymatic antioxidant defense system. Allaouchiche et al.¹⁹ compared propofol (8 mg/kg/h), desflurane (10%) and sevoflurane (2.5%) to determine oxidative situation in pigs. Pigs were exposed to the anesthetic agents for about 120 min; propofol increased glutathione peroxidase levels (GSH-Px) significantly in both bronchoalveolar lavage (BAL) fluid and in circulation. Desflurane caused a significant decrease of GSH-Px in both BAL fluid and circulation, while in the sevoflurane group there were no significant changes identified in BAL fluid and in circulation. They stated that oxidative stress due to desflurane anesthesia might be related to extreme increases in proinflammatory cytokines in alveolar macrophages. Sivaci et al.²⁰ used sevoflurane or desflurane on patients undergoing laparoscopic surgery and observed that both agents had cytotoxic effects due to free radical formation. Desflurane changes oxidative stress and antioxidant mechanisms negatively; they stated that the nitrogen mixture used with desflurane might increase this effect even more. Sivaci et al. determined that desflurane reduced serum GSH levels. However we did not identify any effect on serum GSH levels due to either desflurane or propofol.

Propofol has been found to increase the fluidity of the erythrocyte membrane preventing hemolysis, similar to vitamin E, and shows antioxidant activity. Propofol protects erythrocytes from oxidative and physical stress. Ascorbic acid has been shown to make this effect more pronounced. Inversely volatile anesthetics reduce erythrocyte membrane fluidity inducing hemolysis.²¹ In our study in the propofol infusion group oxidative stress after the operation was reduced compared to values before operation, though not significantly, while at the same time antioxidant capacity was statistically increased after operation. In all three groups statistically significant changes in glutathione peroxidase levels were not observed. In the propofol infusion group in the postoperative period they were a little reduced, in the sevoflurane group in the postoperative period they were a little increased while in the desflurane group no changes were observed. In our study in both sevoflurane and propofol infusion groups TAS values after operation were statistically significantly increased compared to values before surgery.

Conclusion

This study gives an idea that in general anesthesia, necessary for a variety of reasons, in cases under oxidative stress the agent that will least damage the antioxidant system, an important cascade in the humoral defense system, and that will have least effect on the immune system should be chosen. Further research into the immune situation of patients receiving general anesthetic and the relationship between oxidant/antioxidant systems are required.

Conflicts of interest

The authors declare no conflicts of interest.

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