

Effects of Halothane and Isoflurane Anaesthesia on Antioxidant Enzymes in Dogs

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Abstract: Free radicals, along with the impairment of biological membranes and cellular structures and increased biological activity, come out with the results of the changes in the activity of the enzymes followed by the damages of cellular function and metabolism. This condition was attributed to the initiation effects of anaesthetics and other drugs which are used throughout the general anaesthesia. General anaesthesia which is constructed with using many drugs which have various physicochemical structures is a condition affecting lipid peroxidation in the organism directly or indirectly. In the frame of the study, the effects of halothane and isoflurane used in the anaesthesia on Malonyldialdehyde (MDA) concentration were investigated. The study was carried out on 14 dogs with various breeds, ages and sexes which were admitted to Kirikkale University, Faculty of Veterinary Medicine and with various signs of complaint and determined as healthy according to the clinical and haematological examination. In the experiment, halothane (1-2.5%) and isoflurane (1-3%) were investigated as the anaesthetic substances. Diazepam (0.3 mg kg⁻¹, IM) was administered as preanesthesia and thiopental sodium (10 mg kg⁻¹, IV) was administered for the induction of anesthesia to dogs. Halothane and isoflurane were administered to the dogs in the first and second group, respectively. The duration of anaesthesia was determined as 60 min. Five blood samples were taken from the animals at the time prior to premedication, immediately before the gas anaesthesia, after 1 h of the initiation of anaesthesia, 3 and 24 h after the end of anaesthesia into the heparinated tubes. Malonyldialdehyde levels were measured in plasma. During the experimental period, body temperatures, heart rate, respiration rate and blood oxygen saturation values were recorded. Malondialdehyde levels measured during the halothane anaesthesia were numerically lower than those determined in isoflurane anaesthesia in same time period. However, they reached the same levels prior to the anaesthesia within 24 h in both groups. The differences between the two groups were statistically insignificant ($p>0.05$). Additionally, there were no significant differences in body temperature, heart rate, respiration rate and blood oxygen saturation values between the anaesthetic drugs. Results of the comparisons of time and drug type showed that anaesthesia procedures using both halothane and isoflurane did not have any negative effect on the oxidative metabolism in dogs.

Key words: Anaesthesia, dog, halothane, isoflurane, malonyldialdehyde, saturation

INTRODUCTION

Free radicals, together with the disintegrity of biological membranes and cellular structures come out with the results of the changes in enzyme activities followed by the damages of cellular function and metabolism. This condition was attributed to the initiative effect of some anaesthetic agents and drugs which were used throughout general anaesthesia on oxidation (Khinev and Dafinova, 1993). General anaesthesia can impair immunological defence mechanisms and release

inflammatory mediators and free Oxygen Radicals (OFRs) (Goode *et al.*, 1995). Damage to membrane lipids by free radicals is implied by the appearance of lipid peroxidation products during general anaesthesia (Halliwell *et al.*, 1992; Kotani *et al.*, 1995). In this respect, it is important to know whether an anesthetic or a sedative drug has antiradical properties and this feature provides a potential benefit in critically ill patients. Some anesthetic agents were investigated in the sense of antioxidative effects and effects on OFRs production (Murphy *et al.*, 1992; Davidson *et al.*, 1995; Chinev *et al.*, 1995; Weiss *et al.*,

1997). It was indicated in many researches that phagocytic and cytotoxic activities of alveolar macrophages were suppressed by using volatile anaesthetic agents in animals. In a research in which oxidative stress were investigated, it was observed that desfloran increased the MDA concentration and increase in glutathione peroxidase activity however sevofloran did not induce the production of free oxygen radicals in sera and bronchoalveolar lavage taken from pigs (Allaouchiche *et al.*, 2001). It was indicated that free radicals were formed by metabolized halothane via NADPH-sitokrom P-450 system and produced free radicals initiates lipid peroxidation in cell membranes. It was also emphasized that isoflurane which is less metabolized and produces few amounts of free radicals is less toxic than halothane. This hypothesis was supported by the research in which the damages of anaesthesia with halothane and isoflurane in blood cells and its effect on lipid peroxidation and antioxidant enzymes were investigated (Yesilkaya *et al.*, 1998). Halothane, one of the volatile anaesthetic agents used for general anaesthesia has the structure of 2-bromo-2-chloro-1,1,1-trifloroetan is a clear and sweet odor fluid. It is absorbed rapidly throughout respiratory system and accumulated in especially connective tissue. Few amount of it subject to biotransformation.

It is converted to the trifluoroacetic acid from trifluoroacetyl aldehyde as forced to the oxidative non-halojenization with metabolic enzymes. Additionally, organic bromide and chloride are released. Halothane is converted to difluorobromochloroethylene by facing to oxidative defluorination in a very small level and by losing one of its fluor atom.

This substance leads to the some of the unwanted side effects of this drug as it is bound the phospholipids covalently in the cells. Isoflurane has the structure of 1-chloro-2,2,2-trifluoroethyl difluoromethylether is an isomer of enflorane. As very few amount of this drug subject to biotransformation, very small amount of fluor and trifluoroacetic acid produced. The amounts of these substances are not enough to cause a cellular damage (Kaya, 2000). The aim of this study was to investigate the production of possible free radicals and lipid peroxidation before, during and after the anaesthesia of healthy dogs with halothane and isoflurane.

MATERIALS AND METHODS

Animals and experimental procedures: This experiment was conducted on 14 mongrel, 10-36 month aged dogs admitted to the clinics of Kirikkale University, Faculty of Veterinary Medicine of for various reasons and required to have anaesthesia and determined to be healthy according to the clinical and hematologic inspection. Dogs were divided into two groups randomly. Body temperatures, respiration and hearth rates and blood

oxygen saturations values were recorded in all dogs before and during anaesthesia. Protocol of the experiment was approved by The Animal Health Care Committee of the Faculty of Veterinary Medicine in Kirikkale University (10/21).

Induction and inhalation anaesthesia procedures:

Following placement of a catheter in cephalic vein, 0.3 mg kg⁻¹ diazepam (Diazem ampul, DEVA, Istanbul, Turkey) was administered for premedication. Intubation was performed after 10 mg kg⁻¹ thiopental (Pental sodium, I.E.Ulagay, Istanbul, Turkey) was administered for induction of anesthesia. Each animal was randomly assigned to two groups of seven animals each. They were considered to be healthy based on physical and haematological examination. Anaesthesia was maintained with halothane (Halotan, Hoechst, Istanbul, Turkey) in the first group and isoflurane (Isoflurane, Adeka, Samsun, Turkey) in the second group. The fresh gas flow was 2 l min⁻¹. Halothane (1-2.5%) and isoflurane (1-3%) were administered in 100% oxygen. Heart rate, respiration rate, oxygen saturation (SpO₂) and body temperature were recorded (Peta°, KMA 800) during anesthesia. The clinical assessment of the depth of anaesthesia was evaluated by the anesthetist as presence of eyelid, pedal and anal reflexes and increase in the jaw tone. The anaesthesia was maintained with spontaneous ventilation during 1 h, afterwards all dogs were recovered.

Activity of antioxidant enzymes: About five blood samples from each dog; prior to premedication, just before gas anaesthesia administered and 1, 3 and 24 h after the anaesthesia were taken into the lithium-heparinized vacuum tubes (Venosafe, Terumo Europe N.V. Leuven, Belgium) and transferred to the laboratory. Plasma and erythrocytes were separated by centrifugation at 3000 rpm for 15 min (+4°C) immediately and the erythrocytes were stored at -70°C until enzyme assays were performed. Malondialdehyde levels in the plasma samples were determined spectrophotometrically by reaction with 2-Thiobarbituric Acid (TBA) as described by Yoshioka *et al.* (1979).

Statistical analysis: When the main effects of the means between the treatment groups or between different times were significant then the pairwise comparisons of LSD means separation was administered. p<0.05 was considered to be significant, unless otherwise noted.

RESULTS AND DISCUSSION

In this study, the effects of halothane and isoflurane on malondialdehyde levels were investigated. Malondialdehyde levels were measured in the plasma of the blood samples taken before premedication, after

Table 1: Malondialdehyde levels of halothane and isoflurane groups (nmol L⁻¹)

Drug	Prior to diazepam	After thiopental administration	1 h after the administration of volatil anesthesia	3 h after administration of volatil anesthesia	24 h after administration of volatil anesthesia
Halothane	10.38±3.41 (3.11-14.77)	8.90±3.06 (3.57-12.75)	8.66±3.66 (3.57-15.70)	9.19±3.52 (2.48-16.94)	10.34±4.52 (3.11-23.79)
Isoflurane	10.93±3.88 (6.22-19.28)	9.63±3.55 (4.66-17.10)	10.13±2.53 (6.37-13.99)	9.80±2.79 (4.19-14.61)	10.10±6.24 (1.71-31.10)
p-values	0.67	0.53	0.22	0.61	0.83

Table 2: Body temperature, respiration and heart rate values of the animals before and during anaesthesia

Time (min)	Drug	Body temperature	p-value	Respiration	p-value	Heart rate	p-value
Prior to anesthesia	Halothane	37.68±0.46	0.16	31.28±2.75	0.05	87.71±5.12	0.07
	Isoflurane	37.94±0.18		29.57±1.39		83.00±2.88	
10	Halothane	37.65±0.47	0.13	18.71±2.13	0.15	83.28±4.19	0.54
	Isoflurane	37.92±0.18		17.42±2.07		84.85±3.33	
20	Halothane	37.61±0.47	0.16	14.85±0.69	0.42	84.71±3.63	0.95
	Isoflurane	37.87±0.21		14.14±2.03		84.57±3.86	
30	Halothane	37.51±0.44	0.16	14.71±1.49	0.15	86.00±5.85	0.91
	Isoflurane	37.77±0.21		13.42±1.27		85.71±4.99	
40	Halothane	37.45±0.40	0.31	12.85±1.21	0.52	87.00±5.56	0.78
	Isoflurane	37.64±0.19		13.28±1.11		87.71±4.15	
50	Halothane	37.35±0.40	0.27	12.42±1.61	0.33	83.57±7.76	0.01
	Isoflurane	37.55±0.19		13.28±0.95		90.14±4.29	
60	Halothane	37.28±0.39	0.27	12.57±1.81	0.63	85.14±6.03	0.03
	Isoflurane	37.48±0.19		13.00±1.52		90.57±3.90	

induction and 1, 3 and 24 h after volatile anaesthesia administered. It was determined that MDA levels during the anaesthesia were decreased in both anaesthesia groups when compared with prior to the anaesthesia. Additionally, it was determined that MDA levels at 24 h after anaesthesia were elevated to the levels prior to the anaesthesia in both anaesthesia groups (Table 1).

Additionally, body temperature, respiration and heart rates were recorded at before anaesthesia and 10, 20, 30, 40, 50 and 60 min of the anaesthesia (Table 2).

The researchers have been reported that some hypnotics and sedatives had antioxidant properties (Krumholz *et al.*, 1995; Weiss *et al.*, 1997). Propofol, thiopental, midazolam and ketamine at clinical plasma concentrations have minimal effects on OFRs production (Davidson *et al.*, 1995). Krumholz *et al.* (1995) investigated the effects of thiopental, etomidate, ketamine and midazolam on the generation of superoxide anion and hydrogen peroxide by polymorphonuclear leukocyte in vitro. Thiopental inhibited superoxide anion as well as hydrogen peroxide production. Neither etomidate nor ketamine influenced, midazolam suppressed superoxide anion generation but only if a concentration far beyond clinical relevance was used.

In another study, midazolam is reported to decrease superoxide anion production in doses higher than those of clinical use (Krumholz *et al.*, 1995). However, it was found ineffective by the others (Weiss *et al.*, 1997; Erol *et al.*, 2002).

Only thiopental and propofol have efficient oxygen scavenging properties (Weiss *et al.*, 1997). Superoxide dismutase is a prominent defence against the lipid peroxidation products during general anaesthesia

(Mercer *et al.*, 1994; McCord, 2000). Nitrous oxide, fentanyl and droperidol increase lipid peroxidation in rat liver (Chinev *et al.*, 1995).

Propofol, thiopental, midazolam and ketamine at clinical plasma concentrations have minimal effects on OFRs production (Davidson *et al.*, 1995). More recent studies have tried to show the potential antioxidant activity of other anaesthetic agents such as propofol, midazolam, ketamine and vecuronium (Kang *et al.*, 1998; Tsuchiya *et al.*, 2002). These researches are commonly associated with MDA measurement during different surgery procedures such as ischemia and reperfusion injury. At the present time, there are only a few reports concerning the effects of volatile anaesthetics on the antioxidant enzyme activities. Inhalation of volatile anaesthetics under mechanical ventilation induces an inflammatory response. Allaouchiche *et al.* (2001), evaluated the bronchoalveolar and systemic oxidative stress in swine during exposure to propofol, desflurane and sevoflurane. They show that desflurane produces a systemic and a local oxidative stress in comparison with the sevoflurane and propofol. The same researchers also observed that animals exposed to desflurane have increased MDA concentrations and enhanced glutathione peroxidase consumption in serum. Conversely, animals exposed to propofol have lower circulating and local measurements of MDA levels and reduced glutathione peroxidase consumption. Sevoflurane did not induce a chemical reaction leading to the generation of oxygen free radicals. Hence, propofol and sevoflurane were more likely to have antioxidant properties.

In a study conducted by Koksai *et al.* (2004), it was determined that desflurane caused more lipid peroxidation systemically and locally than sevoflurane, in healthy

humans on whom laparoscopic cholecystotomy operation was performed. In a study conducted on dogs to investigate the effects of anaesthetic agents on liver, it was indicated that both isoflurane and sevoflurane anaesthesia were safer than halothane anaesthesia (Topal *et al.*, 2003). Malondialdehyde is one of the important indicators of oxidative metabolism that it was measured in the course of this study. It was indicated in many scientific studies that there were changes in the activity of the drugs with various doses and anaesthesia types and that they affected oxidative metabolism.

CONCLUSION

In this study, it was determined that halothane and isoflurane did not cause any negative effect on oxidative metabolism under applied anaesthesia procedure.

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