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Effect of Induction Agents on the Antioxidative Activity of Desflurane in Dogs

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Summary: *The objective of this study was to determine the effects of different induction agents on the antioxidative activity of desflurane in dogs. Sixteen healthy male crossbreed dogs, aged between 1 and 2 years and weighing between 16 and 22 kg were equally divided into two groups. Anaesthetic protocols were designed as midazolam + thiopental + desflurane (Thio-Des group) or midazolam + ketamine + desflurane (Ket-Des group). Anaesthesia was induced with 0.3 mg kg^{-1} midazolam + 10 mg kg^{-1} thiopental or 0.3 mg kg^{-1} midazolam + 10 mg kg^{-1} ketamine hydrochloride combinations intraveneously. Anaesthesia maintenance was continued for 70 min(s) with 7-8% desflurane. Blood catalase, superoxide dismutase and hemoglobin activities were measured at control, anaesthesia induction, during the desflurane anaesthesia, one hour after anaesthesia and 24 hours after anaesthesia. In conclusion, thiopental administration in the anaesthesia induction increased superoxide dismutase activity ($p<0.05$), whereas ketamine administration increased the catalase ($p<0.05$) and hemoglobin ($p<0.01$) activities during desflurane anaesthesia. But, these enzymes simultaneously decreased in both groups one day after anaesthesia ($p>0.05$). Therefore, it is possible that induction agents play a significant rol on the antioxidative effects of desflurane anaesthesia.*

Key words: Desflurane, ketamine, thiopental, superoxide dismutase, catalase and dog.

Köpeklerde Desfluranın Antioksidatif Etkisi Üzerine İndüksiyon Ajanlarının Etkisi

Özet: *Bu çalışmanın amacı köpeklerde desfluranın antioksidatif etkisi üzerinde farklı indüksiyon ajanlarının etkisini değerlendirmekti. Çalışmada 1-2 yaş ve 16-22 kg ağırlığında 16 melez sağlıklı erkek köpek iki eşit gruba bölündü. Anestezik protokoller midazolam + thiopental + desfluran (Thio-Des grup) veya midazolam + ketamine + desfluran (Ket-Des grup) olarak tasarlandı. Anestezi indüksiyonu intravenöz olarak 0.3 mg kg^{-1} midazolam + 10 mg kg^{-1} thiopental veya 0.3 mg kg^{-1} midazolam + 10 mg kg^{-1} ketamine hydrochloride kombinasyonu ile başarıldı. Anestezi idamesi 70 dakika % 7-8 desfluran ile sağlandı. Kan katalaz, süperoxit dismutaz ve hemoglobin aktiviteleri kontrol, anestezi indüksiyonu, desfluran anestezisi sırasında, anesteziden 1 ve 24 saat sonra ölçüldü. Sonuç olarak, anestezi indüksiyonunda thiopental verilmesi desfluran anestezisi sırasında süperoxit dismutaz aktivitesini ($p<0.05$), ketamine verilmesi ise katalaz ($p<0.05$) ve hemoglobin ($p<0.01$) aktivitelerini arttırdı. Fakat bu enzimler eşzamanlı olarak anesteziden 24 saat sonra her iki grupta da azaldı ($p>0.05$). Bu nedenle desfluranın antioksidatif etkisinde indüksiyon ajanlarının önemli rol oynadığı söylenebilir.*

Anahtar Kelimeler: Desfluran, ketamine, thiopental, süperoxit dismutaz, katalaz ve köpek.

INTRODUCTION

Oxygen free radicals (OFRs), which include malondialdehyde (MDA), hydrogen peroxide, and hydroxyl radicals, cause oxidative stress (5). The red blood cells (RBCs), convey three major scavenging enzymes to diminish the toxic effects that can be caused by uncontrolled levels of superoxide radical anions (O_2^-) or hydrogen peroxide (H_2O_2). These are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (23*)*. SOD and CAT enzyme activities also help maintain the functional and structural integrity of hemoglobin (Hb) within the oxidative environment of the red blood cell (3). Elevation of these enzyme activities also contribute to oxidative stress, which attenuate antioxidative defense (5). Hb can readily generate or interact with OFRs. Although Hb is the principal oxygen carrier, it can scavenge nitric oxide radical as well (23*)*.

General anaesthesia can impair immunological defence mechanisms and release inflammatory mediators and OFRs (8). Damaging of membrane lipids by free radicals is implied by the appearance of lipid peroxidation products during general anaesthesia (9, 13). In this respect, it is important to know whether an anaesthetic or a sedative drug has antiradical properties, a feature with a potential benefit in critically ill patients. Some anaesthetic agents were investigated in the sense of antioxidative effects and effects on OFRs production (2, 10, 19, 20). Nitrous oxide, fentanyl and droperidol increase lipid peroxidation in rat liver (2).

Some studies have tried to show the potential antioxidant activity of other anaesthetic agents such as propofol, midazolam, thiopental, ketamine and vecuronium (4, 10, 19). In addition to the above-mentioned agents, enflurane in dogs (18), halothane (21) and sevoflurane (19) in rats,

desflurane in rat (5) and swine (1) models have also been reported as lipid peroxidation inducer. These researches are commonly associated with MDA measurement during different surgical procedures such as ischemia and reperfusion injuries. Currently, there are only a few reports concerning the effects of volatile anaesthetics on the antioxidant enzyme activities. A recent study has also reported that desflurane increases plasma SOD concentration during laparoscopic cholecystectomy in human (12), and another study reports that desflurane increases liver CAT and SOD levels (5), thereby, it may be cause systemic and regional lipid peroxidation (5, 12). The effects of desflurane on the CAT, SOD and Hb responses have not been well-understood in normal dogs without exposure surgery. Moreover, there is no controlled study comparing the antioxidant enzyme activities of desflurane regarding with different induction agents in dogs. Therefore, the aim of this study was to compare the effects of different induction agents on the antioxidative activity of desflurane by estimating the blood levels of Hb, CAT and SOD in dogs.

MATERIALS AND METHODS

Animal and experimental procedures: The dogs were supplied from Antakya Municipality Dog Care House. The experimental protocols were approved by the Animal Research Committe of University of Mustafa Kemal, Faculty of Veterinary Medicine. Sixteen healthy male crossbreed dogs, aged 1-2 years and weighing between 16 and 22 kg, were equally divided into two groups in this study. The animals were given only water but no food for at least 12 hours before anaesthesia.

Induction and inhalation anaesthesia procedures: Anaesthetic protocols were designed as midazolam + thiopental + desflurane (Thio-DES group) and midazolam + ketamine + desflurane (Ket-Des group). The dogs were premedicated with atropine sulphate for controlling salivation (Atropan®, Vetas, Turkey) at the dose of 0.04 mg kg^{-1} intramuscularly. After 10 minutes of premedication, anaesthesia was induced with 0.3 mg kg^{-1} midazolam (Dormicum®, Roche, Turkey) + 10 mg kg^{-1} thiopental (Pentotal Sodyum®, Abbott, Turkey) or 0.3 mg kg^{-1} midazolam + 10 mg kg^{-1} ketamine hydrochloride (Ketalar®, Pfizer, Turkey) combinations intraveneously. Following endotracheal intubation, both group animals were mechanically ventilated with 100% oxygen, and anaesthesia maintenance was continued for 70 min(s) with 7-8% DES (Suprane®, Baxter, Germany) utilizing an anaesthesia machine (AMS 200, Ankara, Turkey) with Desflurane Tec. 6 Plus Vaporizer (Datex-Ohmeda, USA).

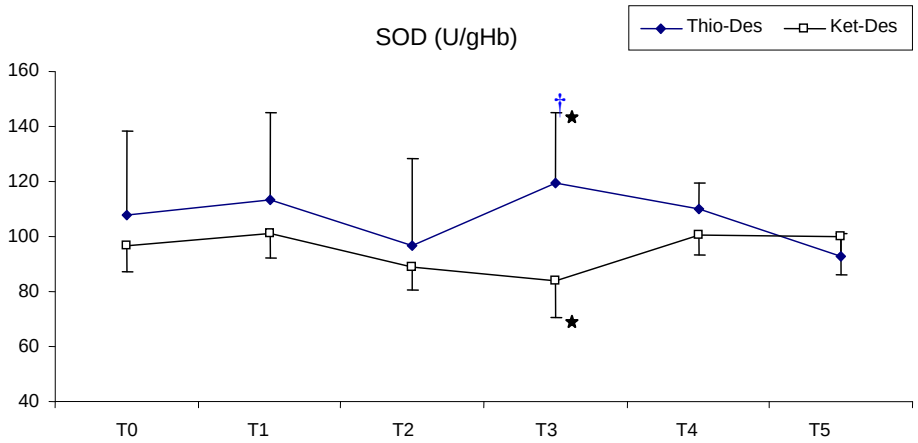
Antioxidant enzyme activities: Heparinized blood samples were collected from dogs by venous puncture at control, anaesthesia induction, 30^{th} and 70^{th} min(s) of desflurane anaesthesia and 1 and 24 hours after anaesthesia respectively. Samples were centrifuged immediately (3.000 rpm, 15 min, 4 °C), the plasma and erythrocytes were separated and these samples were stored at -70 °C until enzyme assays were performed. Superoxide dismutase (SOD) activity and catalase (CAT) activity in erythrocytes were analysed spectrophotometrically as described by Fitzgerald et al. (7) and Luck (15), respectively. Also hemoglobin (Hb) level in blood samples was measured with ferrosiyano Hb method (11).

Statistical analyses: Statistical analyses were accomplished with the use of SPSS computuring programm (version 13.0). Data are reported as means ± standard deviation and analysed with Wilcoxon Signed Ranks Test for the differences within each group and Mann-Whitney Test between groups at each time point. Differences were considered as significant when $p<0.05$.

RESULTS

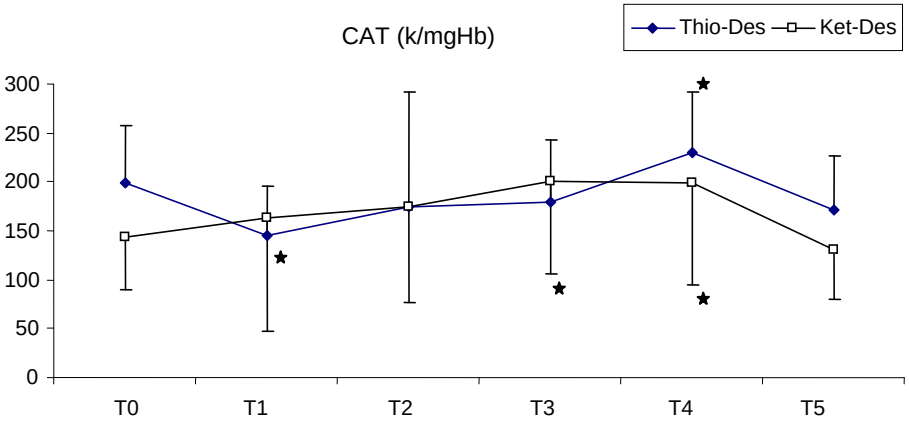
The activities of the blood CAT, SOD and Hb in groups were time-dependently presented in Figure 1, 2 and 3, which show no significant differences between ketamine and thiopental in the CAT, SOD and Hb activities during anaesthesia induction, but significant differences during exposure to Ket-Des and Thio-Des over time in groups. When compared with control (199.66±58.06), CAT activity decreased significantly during anaesthesia induction (145.50±50.87) in the Thio-Des group, whereas, it significantly increased ($p<0.05$) one hour after anaesthesia (229.76±62.42). Compared to control (144.18±53.83), CAT activity in the Ket-Des group non-significantly increased during anaesthesia induction (163.64±116.53) contrary to a significant increase during (200.42±94.65) and one hour after anaesthesia (198.79±103.9). There were no significant differences during the desflurane administration between Ket-Des and Thio-Des groups in the CAT activities ($p>0.05$). On the other hand, when compared with control (107.87±30.25), SOD activity significantly increased during anaesthesia (119.19±25.54) in the Thio-Des group, however, SOD significantly decreased in the Ket-Des group (83.95±13.56) ($p<0.05$). Significant differences were present between two groups during the anaesthesia (70^{th} min) in the amounts of SOD (Figure 1, $p<0.05$). The differences of Hb levels between Thio-Des and Ket-Des groups during and one hour after anaesthesia were found statistically significant (Figure 3, $p<0.01$).

Figure 1. The effect of induction agents on the SOD activity in desflurane anaesthetised dogs.



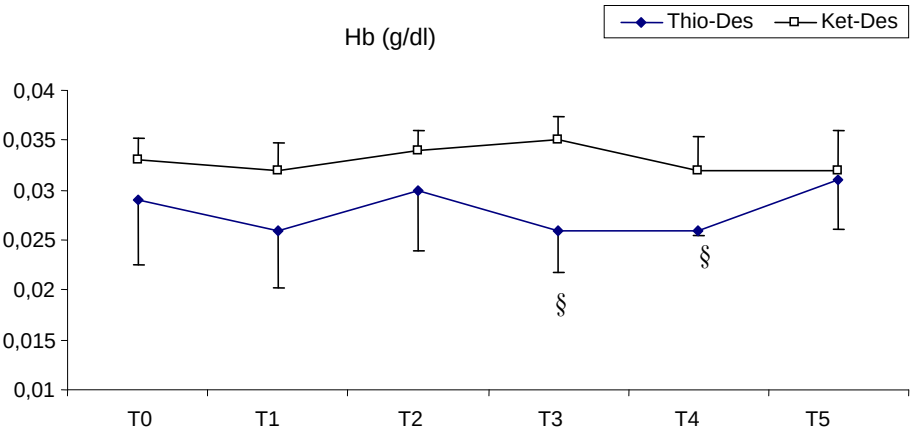
T0: Control, T1: induction, T2: des 30th min, T3: des 70th min, T4: one h after anaesthesia, T5: one d after anaesthesia. †: $P < 0.05$, versus Ket-Des group, *: $P < 0.05$. versus initial value within group.

Figure 2. The effect of induction agents on the CAT activity in desflurane anaesthetised dogs.



T0: Control,T1: induction, T2: des 30th min, T3: des 70th min, T4: one h after anaesthesia, T5: one d after anaesthesia. *: $P < 0.05$ versus initial value within group.

Figure 3. The effect of induction agents on the Hb activity in desflurane anaesthetised dogs.



T0: Control, T1: induction, T2: des 30th min, T3: des 70th min, T4: one h after anaesthesia, T5: one d after anaesthesia. §: $P < 0.01$, versus Ket-Des group.

DISCUSSION

In this study was evaluated the effects of ketamine or thiopental administration on antioxidant activities of desflurane in dogs and the reflections of this alteration on lipid peroxidation. The results demonstrated that there were significant differences in the Hb, CAT and SOD activities during exposure to Ket-Des and Thio-Des over time. Therefore, this study shows that different induction agents may be alter time-dependently the antioxidant effects of desflurane.

The researchers have reported that some hypnotics and sedatives have antioxidant properties (14, 20). Krumholz et al. (14) investigated the effects of thiopental, ketamine and midazolam on the generation of superoxide anion and hydrogen peroxide by polymorphonuclear leukocytes 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 (14). However, midazolam was found ineffective by the others (6, 20). On the other side, it has been that thiopental has efficient oxygen scavenging properties (20). Davidson et al. (4) reported that thiopental, midazolam, and ketamine at clinical plasma concentrations have minimal effects on OFRs production. These results are partially consistent with presented study here. Opposed to the present study, Krumholz et al. (14) reported that thiopental occurred antioxidant effect due to blocking SOD activity. In the present study, it was observed that thiopental and ketamine slightly increased SOD activity during anaesthesia induction (Figure 1, $p>0.05$). SOD provides a prominent defence against the lipid peroxidation products during general anaesthesia (16, 17). It is an essential enzyme detoxifying superoxide radical anions ($O_2^{\cdot}$) to hydrogen peroxide (H_2O_2). Increase in SOD may result in the enhanced formation of H_2O_2. This combined with decrease in catalase activity could lead to increased H_2O_2 accumulation in the tissues and thus inducing oxidative stress (5, 16). Based on the above knowledge, it was seen the existence of a systemic oxidative stress related to thiopental and ketamine administration in this work.

Volatile anaesthetic agents may be the reason for OFRs production by reducing hepatic blood flow. However, these agents may directly elevate and/or suppress the levels of antioxidant enzymes *(*18). Dikmen et al. (5) indicated that sevoflurane might cause more cellular damage than desflurane by inducing the activation of free radical metabolising enzymes at higher rate. Allaouchiche et al. (1) showed that desflurane produced a systemic and a local oxidative stress in swine. However, they found no significant changes for circulating concentrations of SOD during exposure to desflurane. The results of this study suggest that relatively a new volatile anaesthetic agent, desflurane might cause the changes in the antioxidant enzymatic defence system. Production of toxic radical metabolities due to hypoxia, as mentioned by Van-Dyke (22) might be related to increased SOD and CAT enzyme activities. The present study firstly reveals that pretreatment thiopental in dogs exposed to desflurane attenuates the antioxidant defence due to elevation of SOD activity (Figure 1, $p < 0.05$). The Hb levels of Thio-Des group were significantly less than Ket-Des group during anaesthesia (70^{th} min) and one hour after anaesthesia (Figure 3, $p<0.01$). It has been concluded that thiopental is a more potent free radical scavenger on the oxidant stress than ketamine, and that reduction of high oxidation states of Hb may contribute to such activity. It is well documented that auto-oxidation of Hb produces met-Hb and superoxide. Superoxide directly and indirectly, via H_2O_2, leads to further oxidation of Hb and other surrounding molecules (3). Consistent with previous rat (5) and pig (1) animal models (1), the increases of CAT ($p<0.05$) and SOD ($p>0.05$) activities one hour after anaesthesia confirm that desflurane enhances systemic oxidative stress.

In conclusion, thiopental administration in the anaesthesia induction increased SOD activity (Figure 1, $p<0.05$) during desflurane anaesthesia, on the contrary, ketamine administration increased CAT (Figure 2, $p<0.05$) and Hb (Figure 3, $p<0.005$) activities during desflurane anaesthesia. In the light of these findings, it may be said that induction agents play a significant rol on the antioxidative effects of desflurane anaesthesia.

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