

Oxidative Effects of Isoflurane and Medetomidine - Tiletamine / Zolazepam Combination in Beagle Dogs

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Abstract : The present study evaluated the effects of different anesthesia techniques on oxidative stress in beagle dogs. Ten dogs were randomly assigned to either total intramuscular anesthesia with medetomidine-tiletamine/zolazepam (MTZ) combination (group T, 40 µg/kg medetomidine and 2 mg/kg tiletamine/zolazepam) or volatile anesthesia with isoflurane (group I, 2% isoflurane and 100% oxygen). Heart rate, respiratory rate, and rectal temperature for vital signs and the concentration of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) for oxidative stress were measured. SOD activity decreased significantly from baseline anesthesia in both groups ($p < 0.05$). CAT and GPx activities were also decreased significantly after anesthesia between both groups ($p < 0.05$). CAT activity decreased significantly from baseline after anesthesia in both groups, but activities of group I were significantly higher compared with group T after anesthesia ($p < 0.05$). GPx activity in group T decreased significantly from baseline after anesthesia, but activities of group I were significantly higher compared with that of group T 1 hour after the conclusion of anesthesia ($p < 0.05$). In conclusion, general anesthesia seems to induce oxidative stress, and volatile anesthesia with isoflurane attenuates oxidative injuries in beagle dogs.

Key words : isoflurane, medetomidine, tiletamine/zolazepam, antioxidant, oxidative stress.

Introduction

Oxidative stress is defined as the cellular and organ damage caused by reactive oxygen species (ROS) (12). ROS have important roles in various physiological and pathophysiological processes such as inflammation, various diseases, and carcinogenesis (5,13,34). General anesthesia can impair immunologic defense mechanisms (1) and can often result in oxidative injury such as ischemic-reperfusion injury by respiratory depression and blood circulatory disorder (32,37). In addition, oxidative stress metabolites can be produced during anesthesia.

Several agents are used to induce and maintain general anesthesia in veterinary clinics. Injectable anesthetic drugs are often more convenient and economical to use than inhalation anesthetic drugs. But, inhalation anesthetic drugs provide optimal control of anesthesia, rapid induction and recovery from anesthesia, and relatively have few adverse side effects (22). General anesthesia, either with inhalation or nonvolatile anesthetics, affects many organ systems such as cardiovascular and bronchoalveolar systems and the liver (15,16,32,37). Several anesthetic agents produce free radicals and change the serum antioxidant levels in patients. Therefore, antioxidant enzymes scavenge free radicals and prevent their dam-

age. However, the oxidative status of general anesthesia in dogs has not been fully evaluated.

This study investigated the levels of three major antioxidant enzymes - superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) - during anesthesia with nonvolatile (medetomidine-tiletamine/zolazepam, MTZ) and inhalation (isoflurane) anesthetics commonly used in dogs.

Materials and Methods

Experimental animals

Ten clinically healthy beagles with a mean weight of 7.08 kg (6-8 kg) were used in the experiments. These dogs of 2-4 years old comprised two females and eight males. They underwent physical, blood, and radiographic examinations to ensure their health status. Food (Science Diet Adult[®], Hill's Pet Nutrition Inc., USA) was supplied twice daily. Water was supplied as desired. The dogs were fasted for 12 hours prior to experimentation to prevent any possible adverse effects associated with anesthesia. These experimental and housing protocols were approved by the Chungnam National University Animal Care and Use Committee (Approval No. CNU-00043).

Experimental groups

Dogs were randomly assigned to receive the MTZ combination (MTZ) (group T, n = 5) or isoflurane (group I, n = 5). Group T dogs received 40 µg/kg of medetomidine hydro-

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chloride (Domitor[®], Orion Pharma, Finland) and 2 mg/kg of tiletamine-zolazepam (Zoletil[®] 50 mg/ml, Virbac, France) intramuscularly. Group I dogs were each induced by gas anesthesia through a face mask with a Royal-77[®] gas anesthetic machine (Royal Medical, Korea). After intubation, animals were positioned in dorsal recumbency and anesthesia was maintained with 2% isoflurane (Forane[®]; Choong Wae Pharma, Korea) under pure oxygen. Anesthetic gas supply was stopped in the same recovery time (head-up) in Group T and only 100% oxygen was given to enable recovery from anesthesia. The oxygen supply was maintained until the end of the procedure.

Evaluation of vital signs

Heart rate (HR), respiratory rate (RR), and rectal temperature (RT) were measured using a PulsScan-Component patient monitor (Scionic, Korea) and were recorded before anesthesia (0 minutes) and at 10, 20, 30, 40, 50, 60, and 70 minutes after anesthesia with MTZ or isoflurane. During anesthesia, dogs received Hartmann's solution intravenously at a rate of 10 ml/kg/h.

Evaluation of oxidative stress

Blood samples (3 ml) were collected from the cephalic vein at pre-anesthesia (baseline) and at the end of the anesthesia (0 hour) and after the end of the anesthesia at 1 hour. The samples were centrifuged at 3000 rpm for 10 minutes at 4°C to separate plasma and the plasma samples were stored at -80°C until analysis. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) concentrations were measured with commercial kits (Cayman Chemical Company, Ann Arbor, MI, USA) using SynergyTMHT and KC4 ELISA readers (Bio-Tek Instruments, Winooski, VT, USA).

Statistical analysis

Data are expressed as mean \pm SD, and 2-way repeated measures analysis (ANOVA) was used as appropriate. A *p*-value < 0.05 was considered significance. All statistics were

performed using Statistics Package for the Social Sciences, version 17.0 (SPSS, Chicago, IL, USA).

Results

Evaluation of vital signs

HR, RR and RT data are summarized in Table 1. These parameters were not significantly different between both groups. The change of mean HR in group I was greatly increased, compared to before the experiment, and was significantly different at 10, 20, 50 and 60 minutes (*p* = 0.021, 0.016, 0.009, and 0.009, respectively). But, it was decreased (non-significantly) compared with the pre-anesthesia levels in group T. The mean RR was non-significantly different compared with the pre-anesthesia levels in both groups. But, RR of group I was higher than that in group T. The mean RT levels were decreased (non-significantly) compared with the pre-anesthesia levels in both groups. The gas mixture (100% oxygen and 2% isoflurane) was administered for 73 \pm 13.28 minutes (59-92 minutes). Anesthesia induction time was 4.5 \pm 1.73 minutes (1.5-6 minutes) in group T and 5.5 \pm 1.02 minutes (4.5-7 minutes) in group I.

Evaluation of oxidative stress

SOD levels were significantly decreased in both groups compared with baseline values at 0 and 1 hour (group I *p* = 0.04 and 0.03, respectively; group T *p* = 0.04 and 0.03, respectively) (Fig 1). But, there were no significant difference between both groups. CAT and GPx were significantly different between both groups at 0 and 1 hour (CAT *p* = 0.03 and 0.03, respectively; GPx *p* = 0.03 and 0.03, respectively) (Figs 2 and 3). CAT levels in both groups were significantly decreased at 0 and 1 hour (group I *p* = 0.02 and 0.03, respectively; group T *p* = 0.04 and 0.04, respectively) (Fig 2). GPx in group T was significantly decreased in comparison with baseline values at 0 and 1 hour (*p* = 0.04 and 0.04, respectively). Also, in group I, it was significantly decreased at 1 hour (*p* = 0.04) (Fig 3).

Table 1. Vital signs (heart rate, respiratory rate, rectal temperature) in dogs anesthetized with isoflurane or medetomidine-tiletamine/zolazepam combination (MTZ)

| | Group | Pre | 10min | 20min | 30min | 40min | 50min | 60min | 70min |
|------------------------------|---------|------------------|--------------------|--------------------|-------------------|------------------|-------------------|------------------|------------------|
| Heart rate (beats/min) | Group I | 82.4 \pm 27.18 | 103.6 \pm 16.83* | 101.8 \pm 11.21* | 104.2 \pm 10.26 | 97.2 \pm 9.14 | 100.6 \pm 8.29* | 94.2 \pm 5.26* | 95.4 \pm 3.78 |
| | Group T | 90 \pm 13.21 | 68.2 \pm 28.17 | 74.4 \pm 19.17 | 73 \pm 19.27 | 72.2 \pm 19.20 | 65.6 \pm 19.98 | 61.2 \pm 16.99 | 66.4 \pm 14.47 |
| Respiratory rate (beats/min) | Group I | 21.6 \pm 9.10 | 35.6 \pm 31.76 | 33.4 \pm 22.86 | 32 \pm 17.72 | 27.8 \pm 16.25 | 21.8 \pm 11.10 | 28 \pm 16.85 | 29.4 \pm 16.68 |
| | Group T | 21.6 \pm 5.37 | 14.8 \pm 4.82 | 17.4 \pm 4.45 | 13.2 \pm 2.68 | 14.4 \pm 3.29 | 13 \pm 3.46 | 16.4 \pm 8.17 | 18.2 \pm 10.03 |
| Rectal temperature (°C) | Group I | 38.4 \pm 0.26 | 38.2 \pm 0.26 | 38.1 \pm 0.83 | 37.8 \pm 0.55 | 37.7 \pm 0.61 | 37.5 \pm 0.57 | 37.3 \pm 0.56 | 37.1 \pm 0.45 |
| | Group T | 38.6 \pm 0.25 | 38.5 \pm 0.27 | 38.5 \pm 0.33 | 38.4 \pm 0.39 | 38.3 \pm 0.47 | 38.1 \pm 0.52 | 38.1 \pm 0.54 | 38.1 \pm 0.53 |

Data are expressed as mean \pm SD (n = 5).

Group I: Isoflurane group, Group T: Medetomidine-tiletamine/zolazepam combination group.

*Significantly different (*p* < 0.05) from the baseline.

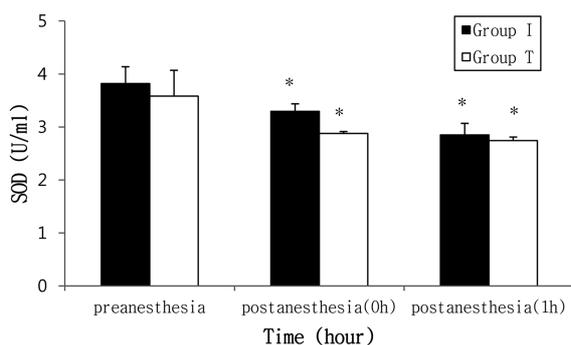


Fig 1. Plasma levels of superoxide dismutase (SOD) in dogs anesthetized with isoflurane or medetomidine-tiletamine/zolazepam combination (MTZ).

Data are expressed as mean \pm SD (n = 5).

Group I: Isoflurane group, Group T: Medetomidine-tiletamine/zolazepam combination group.

*Significantly different ($p < 0.05$) from the baseline.

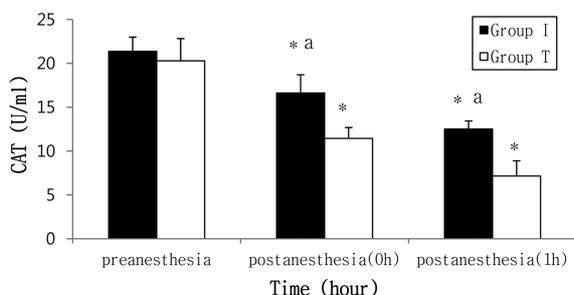


Fig 2. Plasma levels of catalase (CAT) in dogs anesthetized with isoflurane or medetomidine-tiletamine/zolazepam combination (MTZ).

Data are expressed as mean \pm SD (n = 5).

Group I: Isoflurane group, Group T: Medetomidine-tiletamine/zolazepam combination group.

*Significantly different ($p < 0.05$) from the baseline.

^aSignificantly different ($p < 0.05$) from Group T at same time point.

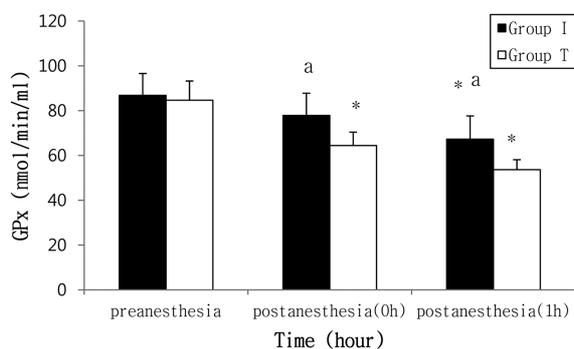


Fig 3. Plasma levels of glutathione peroxidase (GPx) in dogs anesthetized with isoflurane or medetomidine-tiletamine/zolazepam combination (MTZ).

Data are expressed as mean \pm SD (n = 5).

Group I: Isoflurane group, Group T: Medetomidine-tiletamine/zolazepam combination group.

*Significantly different ($p < 0.05$) from the baseline.

^aSignificantly different ($p < 0.05$) from Group T at same time point.

Discussion

This study measured oxidative stress changes as consumption of endogenous antioxidants in anesthesia with injection drugs (MTZ) and inhalation drug (isoflurane) in dogs. HR, RR, and RT were not significantly different between either group. However, the anesthetic used seemed influential in inducing oxidative stress. CAT and GPx activities were significantly different between the groups immediately at the conclusion of anesthesia and 1 hour later. So, volatile anesthesia with isoflurane, rather than MTZ, attenuated oxidative injuries in beagle dogs. CAT levels in both groups and GPx activity in group T were also significantly decreased in comparison with pre-anesthesia values with time. But, GPx activity in group I was only significantly decreased 1 hour after the conclusion of anesthesia.

The intracellular antioxidant SOD catalyzes conversion of superoxide to oxygen and hydrogen peroxide (H_2O_2), and represents the first line of defense against oxygen toxicity (11,12). H_2O_2 is converted to water and molecular oxygen by either antioxidant CAT within the cell membrane or GPx in the cytoplasm and mitochondria (12,13,14,28). Protection of jeopardized organs such as the brain, heart, liver, and lung, as indicated by the SOD, CAT, and GPx values, implicates reduced oxygen intermediates in the anesthesia process, but cannot distinguish free radical species (ROS) (26). ROS occurrence of each regional organ was not examined in this study.

The antioxidative effects of anesthetic drugs, especially propofol and medetomidine, have been reported (23,24,30, 35). Propofol has a lipophilic nature, and so has a good affinity for lipophilic ROS (24,36). Also, dexmedetomidine including α_2 -adrenoreceptor agonist have shown an antioxidant effect on ROS-induced gastric mucosal damage (25). The medetomidine used in this study also is lipophilic in nature and is an α_2 -adrenoreceptor agonist (33). Thus, medetomidine may also have an antioxidant effect. But, the decreased SOD, CAT, and GPx activities in beagle dogs receiving the MTZ anesthesia could reflect the generation of oxidative stress, rather than antioxidant efficacy, by medetomidine, because tiletamine/zolazepam had no antioxidant effects (4). Moreover, the antioxidative effects of isoflurane affect several organs. Antioxidant effects by isoflurane had been reported in focal cerebral ischemia or severe forebrain ischemia of the rat (8,9,26). Isoflurane also can reduce oxidative stress-induced cell injury (21) and protects the liver and heart (10). Besides, myocardial ischemia results in intracellular acidosis and severe hypoxia, subsequently leading to nitrite reduction (38). Isoflurane can prevent peroxidation reactions in heart tissue by scavenging toxic oxygen radicals produced under hyperoxygenation conditions, as occurs with general anesthesia (6). But, isoflurane leads to the impairment of the antioxidant defense system in the guinea pig kidney (7) and isoflurane and halothane increase oxidant-mediated injury in pulmonary artery endothelial cells (27).

The MTZ combination was used presently to reduce

medetomidine and tiletamine/zolazepam adverse effects (18). Side effects of MTZ include hypoxemia, mild hypertension, and acidosis in bears and wood bison anesthesia (2,3). In this study, MTZ behaved very similarly with respect to smooth anesthesia induction and recovery as well as HR and RT results, compared with the pre-anesthesia levels previous studies (18,19). But, the RR difference evident after 20 minutes could be attributable to the different drug dosages in the studies. Also, side effects including hypersalivation were not observed. Isoflurane can change HR according to maintenance doses. In this study, the increase of mean HR as compared with the preanesthesia was similar to the HR changes noted previously (31). Altered RR was noted in both groups presently, but was more pronounced using isoflurane anesthesia.

In general, the temperature reduction in animals sedated with 2-agonists can be attributed to central nervous system depression, in combination with a reduction in muscular activity (29). So, in this study, only slight reductions (no change) in rectal temperature were observed with MTZ. But, RT of group I showed a marked decrease, relative to group T. But, the change was not significantly different between both groups.

Isoflurane does not have direct antioxidant effects (17,20). In this study, the decrease of SOD, CAT, and GPx activities in both groups could mean endogenous antioxidant consumption reduces oxidative stress (oxidants) generated during the anesthetic process or anesthetic metabolism. Therefore, MTZ and isoflurane both form ROS. The CAT and GPx changes between both groups became significantly different with time, with isoflurane producing lower oxidative stress than the MTZ combination.

The collective results support the conclusion that isoflurane anesthesia is less damaging to organs than the medetomidine-tiletamine/zolazepam combination.

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비글 견에서 Isoflurane과 Medetomidine - Tiletamine/Zolazepam 병용의 산화효과

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요 약 : 이 연구는 비글 견에서 산화스트레스에 대한 서로 다른 마취 방법의 효과를 평가했다. 10마리 견들을 무작위로 medetomidine과 tiletamine/zolazepam(MTZ) combination(그룹 T, 40 µg/kg medetomidine and 2 mg/kg tiletamine/zolazepam, IM)을 사용한 근육주사 그룹 또는 Isoflurane(그룹 I, 2% isoflurane and 100% oxygen)을 사용한 휘발성 마취 그룹으로 나누었다. Vital sign으로 심박수, 호흡수, 직장체온과 oxidative stress로 superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)를 측정했다. SOD activity는 두 그룹에서 마취 후 기준 값으로부터 유의성 있게 감소하였다 ($p < 0.05$). CAT와 GPx activity 또한 마취 후 두 그룹 사이에서 유의성이 있었다 ($p < 0.05$). CAT activity는 두 그룹에서 마취 후 기준 값으로부터 유의성 있게 감소하였으나, 그룹 I에서는 마취 후 그룹 T의 그것과 비교 시 유의성 있게 높았다 ($p < 0.05$). 그리고 그룹 T에서 GPx activity는 마취 후 기준 값으로부터 유의성 있게 감소하였으나, 그룹 I에서는 마취종료 후 1 시간이 되었을 때 그룹 T의 그것과 비교 시 유의성 있게 높았다 ($p < 0.05$). 결론적으로, 비글 견에서 전신 마취는 산화 스트레스를 유발시키는 경향이 있었으며, isoflurane의 휘발성 마취는 산화 손상을 감소시켰다.

주요어 : isoflurane, medetomidine, tiletamine/zolazepam, antioxidant, oxidative stress