Evaluation of lipid peroxidation associated with three anesthetic protocols in dogs

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SUMMARY

The oxidative effect of three different anesthetic protocols in dogs by measurement of plasma malondialdehyde (MDA) concentrations before, during and after anesthesia was investigated.

24 mixed-breed dogs with mean body weight 17.4 ± 2.7 kg were divided into 3 experimental groups (n=6 in each), and one control group (n=6). Group 1 was premedicated with atropine sulphate (0.02 mg.kg-1, SC) and acepromazine maleate (0.1 mg.kg-1, IM). Anesthesia was induced with sodium thiopental (10 mg.kg-1, IV) and maintained with 2.5-3 vol % halothane. Group 2 received the same premedication and induction drugs but the maintenance of anesthesia was with fentanyl citrate (0.01 mg.kg-1, IV), halothane (0.5 vol %), pancuronium bromide (0.06 mg.kg-1, IV) and controlled ventilation. Group 3 received the same premedication followed by lumbosacral epidural anesthesia using 2% lidocaine (0.3 mL.kg-1). Group 4 received no drugs but blood samples were collected at the same times as the other 3 groups. Venous blood samples were collected from all animals to determine the concentration of MDA as following : prior to anesthesia (baseline), at the time of premedication (30 minutes), during the deep stage of anesthesia (120 minutes), at the end of anesthesia (140 minutes) and on the next day (24 hours). Blood pressure and core body temperature were measured at each time point using an indirect technique.

Halothane anesthesia was accompanied by statistically increased blood concentrations of MDA at 120 minutes (2.06 ± 0.1 µmol.L-1 ; p<0.05) and 24 hours (2.20 ± 0.19 µmol.L-1 ; p<0.01) in comparison with the baseline (1.61 ± 0.04 µmol.L-1 ; p>0.05) and in comparison with the balanced anesthesia, epidural and control groups. No such changes were seen in the other groups.

Maintenance of anesthesia with halothane alone provoked an oxidative stress that was not manifested with the other anesthetic techniques used in this study.

Keywords : anesthesia - halothane - oxidative stress malondialdehyde - dogs.

RÉSUMÉ

Évaluation du stress oxydatif induit lors de trois protocoles anesthésiques chez le chien. Par G.P. SIMEONOVA, I.I. TODOROVA, V. GADJEVA et D.N. DINEV.

L’Étude a consisté à évaluer le stress oxydatif induit lors de trois protocoles d’anesthésie différents, en mesurant les concentrations plasmatiques en malondialdéhyde (MDA) avant, pendant et après l’anesthésie chez le chien.

24 chiens croisés (poids moyen 17.4±2.7 kg) ont été répartis en 4 groupes de 6 animaux, dont un groupe témoin. Dans le groupe I, les animaux ont été prémédiqués au sulfate d’atropine (0.02 mg.kg-1, SC) et au maléate d’acépromazine (0.1 mg.kg-1, IM), induits au thiopental sodique (10 -1IV) et maintenus avec de l’héxtalane (2.5-3 vol%). Le groupe II a reçu les mêmes agents pour la prémédication et l’induction mais l’anesthésie a été conduite au citrate de fentanyl (0.01 mg.kg-1, IV) et une ventilation contrôlée. Les animaux du groupe III ont reçu la même prémédication suivie d’une anesthésie épidurale lombo-sacrée à l’aide d’une solution de lidocaine à 2% (0.3 mL.kg-1). Ceux du groupe IV n’ont reçu aucune médication ; des échantillons de sang ont été prélevés aux mêmes échéances que les animaux des trois autres groupes.

Les échantillons de sang veineux ont été utilisés pour mesurer la concentration de MDA dans les conditions suivantes : avant l’anesthésie (ligne de base), lors de la prémédication (30 minutes), durant la phase d’anesthésie profonde (120 minutes), à la fin de l’anesthésie (140 minutes) et le jour suivant (24 heures). La pression sanguine et la température centrale ont été mesurées à chaque fois par des techniques indirectes.

L’anesthésie à l’héxtalane entraîne une augmentation statistiquement significative des concentrations sanguines de MDA à 120 minutes (2.06 ± 0.1 µmol.L-1 ; p<0.05) et 24 heures (2.20 ± 0.19 µmol.L-1 ; p<0.01) par rapport aux concentrations de base mesurées avant l’induction (1.61 ± 0.04 µmol.L-1 ; p>0.05), et par rapport aux divers protocoles d’anesthésie épidurale, témoin. Aucune autre modification n’a été retrouvée dans les autres groupes. Cela confirme qu’une anesthésie à l’héxtalane induit un stress oxydatif qui ne se retrouve pas avec les autres techniques anesthésiques utilisées dans cette étude.


Introduction

The production of reactive oxygen species (ROS) in low concentrations is absolutely necessary for some physiological processes such as cell differentiation and proliferation, apoptosis, and cell-mediated immunity [9, 15]. ROS are generated during oxygen metabolism in mitochondrial respiratory chains, in oxidation of hemoglobin and in respiratory burst in granulocytes during phagocytosis [16]. Many exogenous factors such as increased radiation background, increased or decreased partial pressure of oxygen in blood, chemicals and drugs could be sources of ROS [12].

ROS play an important role in the pathogenesis of different diseases [5, 13, 22]. Every increase over their normal concentrations is connected with dangerous toxic reactions which directly affect the cell framework and function [7, 11]. Systemic enzyme and non-enzyme antioxidant defenses maintain ROS concentrations in the physiological range. Oxidative stress occurs when the balance between free radicals and antioxidants is disturbed. ROS cause cellular destruction by oxidative damage of DNA, proteins, lipids and carbohydrates, changing the structure and function of key cellular constituents that results in mutation, cell damage and death. The peroxidation of membrane phospholipids generates malondialdehyde (MDA) - an end product of lipid peroxidation and a marker of oxidative stress.
There are few studies on the generation and toxic influence of free radicals during anesthesia and the results are contradictory. The aim of the present study was to investigate the changes in systemic oxidation, using three different anesthetic protocols, in dogs by measurement of plasma malondialdehyde concentration.

Materials and methods

The investigation was performed on 24 mixed-breed male and female dogs, aged between 3 and 4 years, mean body weight 17.4 ± 2.7 kg divided into four equal groups of six.

The dogs from the first group were submitted to standard halothane anesthesia. They were premedicated with atropine sulphate (Sopharma - Bulgaria; 0.02mg.kg⁻¹, SC) and 10 minutes later with acpromazine maleate (Combistress®, Kela - Belgium, 0.1mg.kg⁻¹, IM). Thiopental sodium (Biochemie GmbH - Austria, 10mg.kg⁻¹, IV) was given 20 minutes later to induce anesthesia. After endotracheal intubation anesthesia was maintained with 2.5 - 3% halothane (Narcotan®, Leciva - Czech Republic) in oxygen at 2.5-3 L.minute⁻¹. A Fluotec III halothane vaporizer and semi-closed rebreathing circuit were used.

The dogs in the second group received the same premedication and induction but a balanced anesthetic technique was used with maintenance for anesthesia using fentanyl citrate (Stobium®, The Chemical Pharmaceutical Research Institute, Sofia, Bulgaria, 0.01mg.kg⁻¹, IV) every 30 minutes; halothane (0.5%, oxygen flow 2.5-3 L.minute⁻¹), pancuronium bromide (Pavulon®, Troypharm, Bulgaria, 0.06 mg.kg⁻¹, IV and repeating the half of the initial dose after every single spontaneous respiratory movement). Controlled ventilation was used with a mean tidal volume of 340 mL and a respiratory rate of 12 minute⁻¹. At the end of anesthesia, galantamine hydrochloride (Nivalin®, Sopharma, Bulgaria, 10 mg, IV) was administered after four spontaneous respiratory movements to reverse the neuromuscular blockade. A deep plane of anesthesia (III/3) in these two groups was maintained. The end of anesthesia was determined as the moment when the dog became spontaneously recumbent.

After the same premedication the dogs from group 3 received lumbosacral epidural anesthesia. Lidocaine solution (Sopharma, Bulgaria, 2%, 0.3 mL.kg⁻¹) was administered into the epidural space between L7 and S1 using a 22-SWG Tuohy needle.

In all three groups 0.9% physiological solution (5 mL. kg⁻¹.hour⁻¹ IV) was administered to prevent the hypotension accompanying all anesthetic protocols. Anesthesia was maintained for up to 120 minutes in the experimental groups.

From the fourth group (control), blood samples were collected without any drugs or fluids being given, to estimate the effect of blood loss upon oxidative status. Venous blood samples were collected from all 24 animals with 15 mmol EDTA in a 10:1 ratio as follows: prior to premedication (baseline) (minute 0), after premedication but before induction (minute 30), during the deep stage of anesthesia (minute 120), at the end of anesthesia (minute 140) and at 24 hours from minute 0. The concentrations of the products of lipid peroxidation were determined in the plasma using the thio-barbituric acid method [19] by spectrophotometric measurement (λ=532 nm) of MDA in µmol.L⁻¹.

An indirect automated device (Digital Electronic Blood Pressure Monitor FC-150 D, Tokyo, Japan) was used to measurement for systolic arterial pressure (SAP) and diastolic arterial pressure (DAP). The mean arterial pressure (MAP) was calculated from the formula : MAP=DAP+(SAP-DAP)/3 in mm Hg [8].

Core body temperature was measured in all animals during all investigated periods using rectal placed electronic thermometer.

Data are presented as mean ± SD. Two-way analysis of variance (ANOVA) was used to detect statistically significant differences (Statmost for Windows, DataMost Corp. 1994-1995). The effects of two factors were studied: time and anesthetic protocol. Post-hoc comparisons were performed by the least significant difference (LSD) test. Differences at the p<0.05 level were considered as significant.

Results

In the halothane group, MDA concentrations increased significantly at minute 120 - during deep anesthesia (2.06 ± 0.1 µmol.L⁻¹, p<0.05) but the highest concentrations were detected by hour 24 (2.20 ± 0.19 µmol.L⁻¹, p<0.01) vs the beginning of anesthesia (1.61 ± 0.04 µmol.L⁻¹). During the recovery period changes in MDA were insignificant.

The changes in blood MDA concentrations in animals receiving epidural and balanced anesthesia were statistically insignificant during the investigated periods compared to baseline (Table I).

The comparative study of alterations in MDA concentrations between groups revealed statistically significantly higher concentrations in the halothane group (2.06 ± 0.1 µmol.L⁻¹) compared to the epidural (1.60 ± 0.2 µmol.L⁻¹, p<0.05) and the balanced anesthesia group (1.62 ± 0.13 µmol.L⁻¹, p<0.05) during deep anesthesia (120 minutes). The same tendency of change was present at 24 hours in the halothane group (2.20 ± 0.19 µmol.L⁻¹) compared to both the epidural (1.41 ± 0.15 µmol.L⁻¹, p<0.001) and the balanced anesthesia groups (1.50 ± 0.08 µmol.L⁻¹, p<0.001).

The mean arterial blood pressure was decreased in all experimental groups by minute 120 in comparison with the baseline and in halothane and balanced anesthesia group compared to the control (table II). At 24 hours the MAP was higher in the halothane group in comparison with the epidural group.

Hypothermia was present in all anesthesia groups at 120 and 140 minutes in comparison with the baseline and control (table III). In the control group, there were no statistically significant differences for all studied parameters and periods.
Discussion

Each anesthesia provokes a stress reaction accompanied by neurohumoral and metabolic responses with the purpose of maintaining homeostasis. The impact of anesthesia in the development of oxidative stress and consequent postanesthetic complications is poorly investigated in veterinary medicine. Some studies found increased radical generation during general anesthesia with halothane [18] and isoflurane [17] whereas in the other studies it was shown that some anesthetics, such as propofol and thiopentone, had antioxidant properties [1, 20].

The administration of acepromazine, used as premedication drug in all three experimental groups resulted in a weaker and insignificant increase, so it could be speculated that this drug was not a potent inducer of oxidative stress. Our results revealed that during the standard halothane anesthesia, blood MDA concentrations were high. These data are similar to those found in some other studies [6, 25].

The mechanisms of lipid peroxidation in halothane anesthesia are complex. On one hand the hypoventilation and the hypoxia usually accompanying halothane anesthesia could be responsible for ROS generation [4]. According to the same authors the tissue hypoxia depresses the activity of liver superoxide dismutase - an antioxidant enzyme at the frontline of defense. In our study none of the animals were hypoxic, as previously described [23], so there is another reason for ROS generation in halothane anesthesia. Hypothermia, accompanying each anesthesia, can induce oxidative stress as well [3] and aggravates hypoxia. In our

<table>
<thead>
<tr>
<th>Anesthetic protocol (group)</th>
<th>N</th>
<th>0 minute</th>
<th>30 minutes</th>
<th>120 minutes</th>
<th>140 minutes</th>
<th>24 hours</th>
</tr>
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<tbody>
<tr>
<td>Controls</td>
<td>6</td>
<td>1.49 ± 0.44</td>
<td>1.30 ± 0.09</td>
<td>1.27 ± 0.02</td>
<td>1.44 ± 0.23</td>
<td>1.68 ± 0.17</td>
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<td>6</td>
<td>1.61 ± 0.10</td>
<td>1.69 ± 0.28</td>
<td>2.06 ± 0.25</td>
<td>*#•</td>
<td>1.73 ± 0.20</td>
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<td>II - Balanced anesthesia</td>
<td>6</td>
<td>1.46 ± 0.11</td>
<td>1.64 ± 0.29</td>
<td>1.62 ± 0.32</td>
<td>1.75 ± 0.32</td>
<td>1.50 ± 0.20</td>
</tr>
<tr>
<td>III - Epidural anesthesia</td>
<td>6</td>
<td>1.42 ± 0.25</td>
<td>1.48 ± 0.36</td>
<td>1.60 ± 0.40</td>
<td>1.75 ± 0.37</td>
<td>1.41 ± 0.36</td>
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</tbody>
</table>

Table I.—Plasma malondialdehyde concentrations in dogs submitted to three anesthetic protocols measured at five periods. The data are presented as mean ± standard deviation. N=number of animals. *p < 0.05; ** p < 0.01 vs baseline; #p < 0.05; ## p < 0.01; ### p < 0.001 vs balanced anesthesia; •p < 0.05; ••p < 0.01; •••p < 0.001 vs epidural anesthesia; ♣p<0.05; vs control.

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<th>120 minutes</th>
<th>140 minutes</th>
<th>24 hours</th>
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<tr>
<td>Controls</td>
<td>6</td>
<td>90±17</td>
<td>92±14</td>
<td>92±9</td>
<td>92±14</td>
<td>87±14</td>
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<tr>
<td>I - Halothane anesthesia</td>
<td>6</td>
<td>99±22</td>
<td>89±23</td>
<td>61±17**</td>
<td>▲▲▲</td>
<td>92±12</td>
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<tr>
<td>II - Balanced anesthesia</td>
<td>6</td>
<td>98±22</td>
<td>76±14</td>
<td>66±18**▲▲</td>
<td>▲▲▲</td>
<td>88±19</td>
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<tr>
<td>III - Epidural anesthesia</td>
<td>6</td>
<td>96±19</td>
<td>84±15</td>
<td>76±17*</td>
<td>80±22</td>
<td>85±11</td>
</tr>
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</table>

Table II.—Mean arterial blood pressure in dogs submitted to three anesthetic protocols measured at five periods. The data are presented as mean ( standard deviation. N=number of animals. *p < 0.05; ** p < 0.01 vs baseline; •p < 0.05 vs epidural anesthesia ; ♣p<0.05; ♣♣p<0.01; ♣♣♣p<0.001 vs control;

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<tr>
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<td>39.1±0.8</td>
<td>39.2±0.7</td>
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<td>I - Halothane anesthesia</td>
<td>6</td>
<td>39.5±0.5</td>
<td>38.8±0.9</td>
<td>36.6±0.7***</td>
<td>▲▲▲</td>
<td>36.3±1.0***</td>
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<td>II - Balanced anesthesia</td>
<td>6</td>
<td>39.1±0.7</td>
<td>38.6±0.9</td>
<td>37.0±0.7**▲</td>
<td>▲▲▲</td>
<td>36.5±0.6**▲</td>
</tr>
<tr>
<td>III - Epidural anesthesia</td>
<td>6</td>
<td>39.0±0.6</td>
<td>39.1±0.5</td>
<td>37.6±0.4*▲</td>
<td>37.2±0.7*▲</td>
<td>38.9±0.4</td>
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</tbody>
</table>

Table III.—Core body temperature in dogs submitted to three anesthetic protocols measured at five periods. The data are presented as mean ( standard deviation. N=number of animals. *p < 0.05; ** p < 0.01 vs baseline; ♣p<0.05; ♣♣p<0.01; ♣♣♣p<0.001 vs control;
study hypothermia was recorded in all anesthesia groups at 120 and 140 minutes but it only corresponded with the increased MDA concentrations during deep halothane anesthesia. Hypoxia is a less strong inducer of lipid peroxidation than halothane resulting in increased MDA concentrations but these two effects accumulate during the anesthesia [25].

On the other hand the stimulation of pituitary-adrenal axis in a stress condition such as anesthesia could be a key factor for the generation of free radicals. A close relationship has been shown between the renin-angiotensin system and ROS generation [24].

Halothane-induced oxidative stress could be directly due to the pharmacological effects of this drug. There is evidence that the liver toxicity of halothane is due to lipid peroxidation in the membranes of hepatocytes. The enhanced peroxidation during halothane anesthesia was explained by anaerobic dehalogenation of halothane in the liver. The obtained anaerobic derivatives of halothane had free radical activity [6]. The lower values during the recovery from anesthesia could be explained by the preliminary cessation of halothane flow and the effects of antioxidant system, which did not last because the blood MDA concentrations at hour 24 were increased again.

The lower MDA concentrations in balanced anesthesia vs those in the halothane group were probably due to the lower concentration of the halothane (0.5 vol%) used in balanced anesthesia. The exposure of 0.5% halothane anaesthesia for 45 minutes does not induce oxidative stress [20] while the same concentration after 12 hours duration of anesthesia causes lipid peroxidation [21].

Epidural anesthesia did not have any significant effect on the generation of ROS.

The hypotension recorded during deep stages of the three anesthetic protocols only corresponded with the increased MDA concentrations in the halothane group. Therefore, the low blood pressure did not influence oxidative stress in these schemes of anesthesia. There is not direct evidence that oxidative stress could be due to low cardiac output. We reject this hypothesis on the grounds of some literature data. Propofol causes a significant decrease in mean arterial pressure, systemic vascular resistance and cardiac output [10] but it is also known to have antioxidant properties [1]. Epidural applied lidocaine results in sympathetic blockade and increase in vagal reflexes but it decreases plasma MDA concentrations as well [2, 14].

In conclusion, oxidative stress was manifested by significantly increased blood MDA concentrations in halothane-anesthetized dogs at 120 minutes and 24 hours. In the other anesthetic techniques such changes during the same periods did not occur.

References

24. — YAMAZOE K., INABA T., BONCOBARA M., MATSUKI N., ONO K., KUDO T.: Changes of hepatic tissue phospholipid peroxida-