Antioxidant capacity of lipid- and water-soluble antioxidants in dogs with subclinical myxomatous mitral valve degeneration anaesthetised with propofol or sevoflurane

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SUBJECT AREAS

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Abstract
Background
Antioxidants located in both the hydrophilic and lipophilic compartments of plasma act as a defence system against reactive oxygen species (ROS), which are continuously generated in the body due to both normal metabolism and disease. Excessive production of ROS during anaesthesia affects the antioxidant capacity of plasma and may result in oxidative stress. The aim of this study was to evaluate the antioxidant capacity of lipid- (ACL) and water-soluble (ACW) antioxidants in client-owned dogs diagnosed with periodontal disease and myxomatous mitral valve degeneration (MMVD) and anaesthetised for a dental procedure with propofol and sevoflurane or with propofol only.

Results
Dogs with MMVD were anaesthetised with propofol and sevoflurane (MMVD/PS, n = 8) or with propofol only (MMVD/P, n = 10). Dogs with no evidence of MMVD (PS, n = 12) were anaesthetised with propofol and sevoflurane. Blood samples for determination of ACL and ACW were collected before and 5 minutes, 60 minutes and 6 hours after induction to anaesthesia. In dogs with MMVD and anaesthetised with propofol and sevoflurane, ACL was significantly higher at all sampling times when compared to control dogs. In dogs with MMVD and anaesthetised with propofol, ACW increased after induction to anaesthesia and remained elevated up to 6 hours after anaesthesia. The only difference between propofol and propofol/sevoflurane anaesthesia in dogs with MMVD was significantly higher ACW at 60 minutes after induction to anaesthesia in the propofol group.

Conclusions
Compared to basal values, anaesthesia maintained with either propofol or sevoflurane significantly increased ACW in both dogs with and without MMVD. In dogs with MMVD, ACW was significantly higher in the propofol group. Furthermore, only anaesthesia with propofol significantly increased ACL in dogs with MMVD. These results suggest that regarding antioxidant capacity, propofol might be a better choice than sevoflurane for anaesthesia in dogs with MMVD.

Background
The heart is constantly subjected to ROS formation due to the high rate of aerobic metabolism [1].
Antioxidants located in both the hydrophilic and lipophilic compartments of plasma are actively involved in a defence system against ROS [2]. Acute and chronic stress may disrupt the equilibrium between ROS and antioxidant mechanisms and increase oxidative stress, which may eventually lead to deleterious structural and functional changes in the heart [3]. In dogs with MMVD, oxidative stress may be implicated in the pathogenesis and progression of the disease, although the exact mechanisms are still unknown [4, 5, 6]. Increased formation of ROS during anaesthesia [7] may worsen MMVD in dogs [8].

Propofol and sevoflurane are commonly used anaesthetics in dogs. Propofol has a structural feature, the phenolic hydroxyl group, like vitamin E, and is believed to act as a ROS scavenger [9, 10]. Sevoflurane, on the other hand, may promote ROS formation through its metabolism [11] and by influencing mitochondrial function [12].

In order to determine the most appropriate anaesthesia protocol for the dogs with MMVD, we determined the antioxidant capacity of lipid- and water-soluble antioxidants in dogs with MMVD which were anaesthetised with propofol alone, or in combination with sevoflurane, for a dental procedure. We hypothesised that total intravenous anaesthesia with propofol increases ACL and ACW compared with anaesthesia induced with propofol and maintained with sevoflurane in dogs with MMVD.

**Results**

Eighteen dogs diagnosed with MMVD ACVIM class B1 and B2, 7 females (3 neutered, 4 intact) and 11 males (3 neutered, 8 intact), weighing 17.52 ± 8.86 kg and aged 8 years and 8 months ± 3 years were included in the study. The control group consisted of 12 dogs with no cardiac disease, 6 females (all neutered) and 6 males (2 neutered, 4 intact), weighing 23.11 ± 9.08 kg and aged 5 years and 11 months ± 2 years and 5 months.

Some samples for determination of ACW were excluded from the study due to technical problems with latent fibrin formation and the results are reported only for 7 dogs in the MMVD/PS group, 10 dogs in the PS group and 8 dogs in the MMVD/P group.

There were no differences between the groups in periodontal/dental disease status, antibiotic administration, use of regional nerve blocks, age of dogs and duration of anaesthesia. Ketamine was
administered significantly more frequently in dogs with MMVD compared to dogs with no cardiac disease.

ACL values were significantly lower in the PS group compared to those in the MMVD/PS group at all sampling times. Compared to basal values, ACL increased significantly 60 minutes after induction to anaesthesia in the MMVD/P group (Table 1). The ACL value of the propofol formulation used in the study was 3.406 micromoles of trolox equivalents per millilitre of propofol solution containing 56.1 micromoles of propofol in equal quantity.

Compared to basal values, ACW increased significantly 5 minutes after induction to anaesthesia and remained elevated until 6 hours after induction only in the MMVD/P group. At 60 minutes after induction to anaesthesia, ACW was also increased in the MMVD/PS and PS groups; the increase was significantly higher in the MMVD/P group compared to that in the MMVD/PS group (Table 1).

Table 1

| Variable | Group    | Basal       | 5 min       | 60 min      | 6 h        |
|----------|----------|-------------|-------------|-------------|------------|
|          |          | Basal       | 5 min       | 60 min      | 6 h        |
| ACL [nmol/L] | MMVD/PS | 133.9 ± 31.3 | 130.9 ± 29.9 | 134.3 ± 31.9 | 131.8 ± 26.2 |
| ACL [nmol/L] | MMVD/P   | 87.6 ± 12.2  | 86.6 ± 13.7 | 93.0 ± 18.6 | 84.9 ± 22.2 |
| ACL [nmol/L] | PS       | 116.4 ± 22.9 | 118.2 ± 19.9 | 134.0 ± 23.3* | 119.1 ± 24.6 |

Data are presented as (mean ± SD). Abbreviations: basal, basal values; 5 min, 5 minutes after induction to anaesthesia; 60 min, 60 minutes after induction to anaesthesia; 6 h, 6 hours after induction to anaesthesia. * p < 0.05 compared to basal values; a p < 0.05 compared to MMVD/PS.

Cholesterol concentrations before induction to anaesthesia were significantly lower in the PS group compared to those in the MMVD/PS group (Table 2).

Table 2

| Group    | Cholesterol [mmol/L] | Triglycerides [mmol/L] |
|----------|----------------------|------------------------|
| MMVD/PS  | 7.29 ± 1.62          | 1.01 ± 0.64            |
| PS       | 5.42 ± 1.37a         | 0.80 ± 0.57            |
| MMVD/P   | 6.52 ± 0.85          | 0.78 ± 0.2             |

Data are presented as (mean ± SD); a p < 0.05 compared to MMVD/PS.
Discussion

The plasma antioxidant capacity of lipid-soluble antioxidants covers exogenous and endogenous lipophilic antioxidants, including vitamin E, coenzyme Q₁₀ and carotenoids. In the plasma of healthy men, vitamin E represents up to 75% of ACL [13]. Studies suggest that during anaesthesia a decrease in vitamin E (total tocopherol) in dogs with MDMZ [<link rid="bib14">14</link>], healthy dogs [15] and people [16, 17], as well as a decrease in alpha tocopherol in people [18, 19], may be due to the prooxidative activity of inhalation anaesthetics. Hypoxia during anaesthesia is the primary cause of changes in antioxidant defence and malondialdehyde production, while the addition of an inhalation anaesthetic results in increased depletion of reduced glutathione and especially vitamin E [<link rid="bib20">20</link>].

Significantly higher plasma ACL was determined in the MMVD/PS group at all sampling times when compared with the PS group in this study. This might be due to increased mobilisation of vitamin E and other lipid soluble antioxidants in response to enhanced ROS production in dogs with cardiac disease [21, 22]. On the other hand, vitamin E concentrations before anaesthesia may have been influenced by increased cholesterol concentrations found in dogs with MMVD [23], which was also observed in our study.

The significant increase of ACL 60 minutes after induction in the MMVD/P group might be due to the contribution of propofol to ACL. The antioxidant properties of propofol have already been revealed [9, 24, 25, 26]. To assess the possible contribution of propofol to ACL, we measured ACL in a sample of the same propofol formulation as we used in the study and established that the propofol formulation has an extremely high ACL, which confirms its antioxidant properties. This is in accordance with studies of antioxidant capacity in human patients during propofol anaesthesia [18, 27, 28]. The lipid soluble component of blood antioxidant activity was evaluated in healthy women, and although propofol had only a small influence on total antioxidant capacity, there was an increase in the antioxidant protection of lipid membranes [28]. Furthermore, propofol accumulates in lipid membranes; thus, plasma may not be the best compartment to evaluate the antioxidant effect of propofol [27].
The antioxidant capacity of water-soluble antioxidants includes hydrophilic antioxidants such as vitamin C, glutathione, uric acid, proteins and low molecular antioxidants [2, 29]. No differences in basal values of ACW were observed between the MMVD/PS and PS groups, probably because the homeostasis of water-soluble antioxidant mechanisms was not disrupted in dogs with early-stage MMVD. The only difference between the two anaesthesia protocols in dogs with MMVD was at 60 minutes after induction to anaesthesia, when significantly higher ACW was determined in dogs anaesthetised with propofol. Significantly elevated ACW 60 minutes after induction to anaesthesia in all groups, and 6 hours later in the MMVD/P group, may be attributed to the free radical scavenging activity of propofol [9, 25]. Similar results were reported for healthy men anaesthetised with propofol for elective surgery [18].

The limitation of this study is that the dogs were client-owned, and thus the environmental and nutritional backgrounds of the dogs differed, which may have influenced the antioxidant parameters [30]. In fact, antioxidant status may have been affected by different levels of environmental pollution (including for example smoking in the household), and although the owners stated that the dogs were not given any vitamins or antioxidants and were mostly fed commercial food, the composition of their food may have differed in antioxidant and vitamin content.

**Conclusions**

Compared to basal values, ACW was significantly increased 60 minutes after induction to anaesthesia in both dogs with and without MMVD regardless of the anaesthesia protocol. In dogs with MMVD, ACW was significantly higher in the propofol group. In dogs with MMVD, only anaesthesia with propofol significantly increased ACL. These results suggest that regarding antioxidant capacity, propofol might be a better choice than sevoflurane for anaesthesia of dogs with MMVD.

**Methods**

**Animals**

This prospective clinical study was evaluated and approved by the National Ethics Committee and written informed client consent was obtained before the dogs were included in the study. All procedures complied with the relevant Slovenian (Animal Protection Act UL RS, 43/2007) and European regulations. Client-owned dogs receiving no medication one month prior to anaesthesia
were recruited from the University of Ljubljana Veterinary Faculty subject pool. Their health status was evaluated based on history, physical examination and blood tests including complete blood count with white cell differential count and serum biochemical analyses (glucose, urea, creatinine, sodium, potassium, chloride, calcium, total protein, albumin, alanine aminotransferase, alkaline phosphatase, serum total cholesterol and triglycerides). Cardiovascular disease was confirmed by history, clinical examination, standard electrocardiogram and echocardiography using two-dimensional, M-mode, and colour and spectral Doppler modes (VIVID E9, General Electric Healthcare, Milwaukee, Wisconsin, USA).

All eligible dogs that were presented between June 2016 and January 2017 were included. The final sample size was n = 30. The dogs were diagnosed with periodontal disease and scheduled for a dental procedure under general anaesthesia. Eighteen dogs were diagnosed with MMVD class B1 or B2 according to the American College of Veterinary Internal Medicine (ACVIM) classification [31]. They were randomly assigned (by tossing a coin) to anaesthesia with propofol (MMVD/P, n = 10) or sevoflurane (MMVD/PS, n = 8). The control group consisted of 12 dogs with no evidence of MMVD.

**Study Protocol**

Dogs were premedicated with morphine 0.3 mg/kg administered subcutaneously (SC) 20 minutes before induction to anaesthesia. An intravenous catheter was placed into the cephalic vein, and after 5 minutes of preoxygenation, anaesthesia was induced with propofol, which was administered intravenously (IV) and titrated to effect. The dogs were intubated endotracheally, connected to a circle breathing system and allowed to breathe oxygen spontaneously. In dogs with MMVD, anaesthesia was maintained with propofol at 0.3–0.6 mg/kg/min IV (MMVD/P group) or with sevoflurane at an end-tidal sevoflurane concentration of 2 to 3% (MMVD/PS group). Dogs with no evidence of MMVD (PS group) were anaesthetised the same way as the MMVD/PS group. Hartmann's solution was infused at 5 mL/kg/h IV during anaesthesia. Perioperative analgesia was supported with oral regional nerve blocks with levobupivacaine 1 to 2 mg/kg and/or IV boluses of ketamine 0.5 mg/kg. Perioperative antibiotic management was carried out with cefazolin 20 mg/kg IV if clinically indicated.
The dogs were placed in dorsal recumbency and warmed with bags filled with warm water. End-tidal sevoflurane concentration, end-tidal CO\textsubscript{2} tension, respiratory rate, ECG, blood pressure (non-invasively) and rectal temperature were monitored during anaesthesia.

Dogs with the majority of teeth present were included in the study, and a detailed oral examination (probing, charting and full-mouth dental radiographs) was performed prior to periodontal treatment. Dogs were divided into two groups based on their oral/dental disease for statistical analysis: ≤ 25% of the teeth affected with periodontitis and/or dental fractures and > 25% of the teeth affected with periodontitis and/or dental fractures.

During recovery, the dogs were administered Hartmann's solution at 2 mL/kg/h IV and morphine 0.3 mg/kg SC every three hours, depending on the invasiveness of the procedure. Carprofen 4 mg/kg IV was administered after the last blood sampling. All dogs were released to home care the same day, with analgesics and antibiotics prescribed as clinically indicated.

**Blood Sample Collection, Processing And Analyses**

Venous blood samples were collected from the jugular vein before premedication (basal values) and 5 minutes (immediate post induction period), 60 minutes (intraoperative period) and 6 hours after induction to anaesthesia (postoperative period).

Blood samples for determination of ACL and ACW concentrations (total volume 2 mL) were collected in lithium heparin containing tubes (Vacuette, Greiner Bio-One, Kremsmunster, Austria). Samples were immediately centrifuged at 1500 × g for 15 minutes at 4 °C and plasma immediately frozen at -80 °C until analysis. Blood samples (2 mL) for determination of total cholesterol and triglyceride concentrations before anaesthesia were collected into serum separator tubes (Vacuette, Greiner Bio-One, Kremsmunster, Austria). After clotting at room temperature, the samples were centrifuged at 1300 × g for 10 minutes at room temperature and immediately assayed (RX Daytona, Randox, Crumlin, UK).

**Determination Of Antioxidant Capacity Of Water- And Lipid-soluble Antioxidants**

The method is based on the chemiluminometric detection of photochemically generated superoxide anion radicals from a photosensitizer (luminol), which are partially eliminated from the sample by
reaction with antioxidants present in the sample. The remaining radicals react with luminol to produce luminescence, which is measured with a PHOTOCHEM analyser (Analytik Jena, Jena, Germany) using supplied reagent kits. After defrosting and vortexing, 100 µL of plasma sample and 100 µL of methanol were pipetted in a 1.5 mL plastic container with a cap. Samples were then vortexed for 10 minutes and centrifuged at 25,000 rpm for 10 minutes at 4 °C. Sample preparation steps were performed in a dark place. Until analysis (usually within 30 minutes), prepared samples were held in the dark at a temperature below 4 °C. Plasma ACL and ACW were measured in accordance with the manufacturer instructions, using reagent kits (Analytik Jena, Jena, Germany) with the PHOTOCHEM analyser. The results of the ACW measurements are expressed in nmol equivalents of ascorbic acid in mL of the sample, and the results of the ACL measurements in nmol equivalents of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in mL of the sample.

To assess whether the propofol formulation used in the study has antioxidative properties, we also measured ACL in a sample of propofol formulation, using the same method as described above.

**Statistical analysis**

An a priori sample size calculation was not performed as no comparable data from the literature regarding ACW and ACL were available to enable calculation. Post-hoc sample size calculation indicated that with an effect size of 0.5 and significance level \( p = 0.05 \), 30 dogs would be enough to achieve more than 80% power of the study. Statistical analysis was supported by the R statistical software program (version 3.2.2) with the nlme package [32]. The Mann-Whitney test was used to evaluate the differences in the baseline characteristics of the dogs, age and duration of anaesthesia. Fisher’s exact test was used to evaluate the differences between the MMVD/PS, MMVD/P and PS groups with respect to the baseline characteristics of periodontal/dental disease status and the use of antibiotics, ketamine and nerve blocks. A linear mixed-effect analysis was used to examine the treatment effect and trends over time within groups (MMVD/PS, MMVD/P and PS) and between groups (the MMVD/PS group was compared to the MMVD/P and PS groups). The models included a random intercept for each dog and three fixed effects: time, group and interaction term. In case of significant interaction, multiple comparisons were performed using Holm-Bonferroni correction. A value of \( p < \)
0.05 was considered significant.

**Abbreviations**

ACL: Antioxidant capacity of lipid-soluble antioxidants; ACW: Antioxidant capacity of water-soluble antioxidants; IV: intravenously; MMVD: myxomatous mitral valve degeneration; MMVD/P: dogs with MMVD and anaesthetised with propofol; MMVD/PS: dogs with MMVD and anaesthetised with propofol and sevoflurane; PS: dogs with no evidence of MMVD and anaesthetised with propofol and sevoflurane; ROS: reactive oxygen species; SC: subcutaneously

**Declarations**

**Competing interests**
The authors declare that they have no competing interest.

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**Authors’ contributions**
AS and ANS designed the experiment. KT, AN, ADP and AS performed clinical experiments. TP, VR, TV and ANS performed laboratory analyses and interpreted the results. KT, AN, ANS and AS drafted the manuscript. AS and ANS reviewed the manuscript. All authors read and approved the final manuscript.

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**Ethics approval and consent to participate**
All procedures involving the use of animals were approved by the National Ethics Committee (license No U34401-38/2013/2, approval date 30.7.2013), and written informed client consent was obtained before the dogs were included in the study. All procedures complied with the relevant Slovenian (Animal Protection Act UL RS, 43/2007) and European legislation.

**Availability of data and materials**
The data generated or analysed during the current study are available from the corresponding author on reasonable request.

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