Research in Veterinary Science 117 (2018) 92–96

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Serial evaluation of serum total reduction power potential by cyclic voltammetry in 30 dogs with gastric dilatation and volvulus- a randomised, controlled (lidocaine vs placebo), clinical trial

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1. Introduction

Gastric dilatation and volvulus (GDV) in dogs is an acute, life threatening syndrome leading to ischemic reperfusion injury (IRI) (Cassutto et al., 1989). Hemodynamically, GDV is characterized by obstructive and a relative hypovolemic-distributive shock, leading to ischemia (Bruchim and Kelmer, 2014). During gastric decompression and surgical repositioning, reperfusion of hypoxic tissues occurs, leading to IRI and reactive oxygen species (ROS) production. Reactive oxygen species have been implicated in cell and tissue damage, and in activation of the inflammatory system (Ginsburg et al., 1992), leading to some of the common complications in canine GDV. These include systemic inflammatory response syndrome (SIRS), hypotension, acute kidney injury (AKI), disseminated intravascular coagulation and cardiac arrhythmias (Bruchim et al., 2012; Buber et al., 2007; Israeli et al., 2012; Sharp and Rozanski, 2014; Zacher et al., 2010). The mortality rate in GDV is relatively high (10%–28%), despite intensive medical and surgical treatment (Israeli et al., 2012; Zacher et al., 2010). Therefore, in addition to medical stabilization and gastric decompression, prevention and treatment of IRI, and its consequent oxidative stress, should be among the therapeutic goals (Lantz et al., 1992).

Lidocaine is a potent local anesthetic and class Ib anti-arrhythmic agent, acting on fast sodium channels during the inactive phase, inhibiting their depolarization. Intravenous lidocaine has analgesic (Groudine et al., 1998), antihyperalgesic (Koppert et al., 1998), and anti-inflammatory properties (Hollmann and Durieux, 2000), which makes it effective in decreasing IRI and SIRS (Barthel et al., 2004; Cao et al., 2005; Lan et al., 2004). A previous study of an experimentally-induced GDV in dogs demonstrated that pre-ischemic lidocaine treatment reduces gastric and cardiac histopathologic and ultrastructural tissue damage, and occurrence of cardiac arrhythmias (Pfeiffer et al., 1989). Another study demonstrated that an early intravenous lidocaine bolus (2 mg/kg), followed by constant rate infusion (CRI) (0.05 mg/kg/min) for 24 h post-presentation, significantly decreased occurrence of cardiac arrhythmias, AKI and hospitalization time-period (Bruchim et al., 2012). Another recent study has shown better survival rate in dogs with septic peritonitis treated intraoperatively with lidocaine (Bellini and Seymour, 2016). The improved clinical outcome is presumed to be related to the reduction of the inflammatory and oxidative mediators, although a direct measurement of inflammatory mediators, anti-oxidants or indicators of oxidative stress has not been thoroughly evaluated (Bruchim et al., 2012). However, this hypothesis is supported by a study of 13 dogs with GDV demonstrating a significant increase in serum oxidation products (e.g. malondialdehyde), a decrease in total anti-oxidant capacity, as measured by oxygen radical absorbance capacity, and in increase in free radical scavenger concentration (e.g., vitamins C and E) (Walker et al., 2007).

Tissue and body fluids withstand oxidative stress by utilizing endogenous and exogenous anti-oxidant compounds (Ligumsky et al., 2005). The reduction potential of biological fluids and tissues correlates with the overall scavenging capacity of the sample (low molecular weight anti-oxidants (LMWA) activity) (Kohen et al., 1999; Ligumsky et al., 2005), which can be quantified by cyclic voltammetry (CV), a reliable method that evaluates the total reducing power (TRP) of the plasma, determined by the type (peak potential) and concentration (anodic current) (Ginsburg et al., 1992; Kohen, 1993; Kohen et al., 1992; Ligumsky et al., 2005). Changes in TRP are reflected by decrease in the anodic current, indicating decrease in LMWA concentration and/or disappearance of the anodic wave, due to absence of a specific group of LMWA. These changes may indicate a reduced capacity to cope with oxidative stress (Kohen et al., 2000; Ligumsky et al., 2005).

Our hypothesis is that dogs with GDV have decreased TRP, and that lidocaine treatment improves TRP. The aims of this study are: 1) To assess the TRP capacity of dogs with GDV at admission and during their hospitalization period. 2) To assess the association between TRP and disease severity and outcome. 3) To assess lidocaine treatment ability to restore TRP capacity.

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https://doi.org/10.1016/j.rvsc.2017.11.019
Received 31 December 2016; Received in revised form 25 July 2017; Accepted 26 November 2017

2. Materials and methods

2.1. Study design and animals

This study was a prospective, randomised, placebo controlled clinical trial. The study was approved by the Animal Care and Use Committee of the Koret University Veterinary Teaching Hospital. Animals included after their owners have signed a consent form. Dogs presented to the emergency service of the Hebrew University Veterinary Teaching Hospital (HUVTH) and diagnosed with GDV, based on history, physical examination, abdominal radiography and surgical confirmation, were enrolled. Lidocaine and saline 0.9% were prepared in advanced in identical vials and labeled. Labeling code was known only to the chief pharmacists and was disclosed only at the end of the study. Dogs were assigned to either treatment (lidocaine) or placebo (saline) groups by randomly selecting a slip from a sealed envelope.

2.2. Laboratory tests

Blood sampled for complete blood count (CBC) were collected in K3EDTA tubes, and analyzed within 30 min from collection using a commercial analyzer (Abacus or Arcus, Diatron, Wien, Austria; or Advia 120, Siemens Medical Solutions Diagnostics GmbH, formerly Bayer). Blood samples for lactate analysis were collected in fluoride tubes, centrifuged within 30 min from collection, and sera were either analyzed immediately or stored at 4 °C pending analysis, that was performed within 12 h from collection (Cobas-Mira or Cobas-Integra 400, Roche, Mannheim, Germany, at 37 °C).

In order to assess oxidative status, blood samples from animals diagnosed with GDV were collected into heparin tubes at presentation, and 4, 12 and 24 h later. All tubes were protected from light using aluminum foil. Blood was centrifuged (4000 rpm, at 4 °C for 15 min) and sera were stored at -80 °C pending analysis. Eleven healthy dogs, based on intact history, physical examination and CBC, were used as a control group for the assessment of TRP.

2.3. Treatment protocol

Dogs were treated according to the recommendations for management of canine GDV (Sharp, 2014). Upon arrival, immediately after IV catheter placement and before any other medical or surgical intervention, dogs were treated with either lidocaine (2 mg/kg IV) or saline (placebo) at the same volume, followed by lidocaine or saline CRI infusion at 0.05 mg/kg/min, for 24 h. Isotonic crystalloid fluids were administered as bolus at 20 ml/kg IV. Additional treatment with either crystalloid or colloid was administered according to predetermined resuscitative end points (Sharp, 2014). For gastric decompression, dogs were pre-medicated with pethidine (2 mg/kg, SQ), while diazepam (0.5 mg/kg, IV), and fentanyl (5–10 mcg/kg, IV) were used for induction. Gastric decompression was accomplished preferably using orogastric intubation, with or without trocrization. Immediate surgical intervention was performed as soon as cardiovascular stability was reached.

2.4. Cyclic voltammetry

Heparinized plasma was placed in wells containing three electrodes (Fig. 1): a working electrode (a glassy carbon disc), a counter electrode (platinum wire) and a reference electrode (Ag/AgCl). A constant electric potential was applied (100 mV/s) in a positive direction, between the two first electrodes versus the reference one (up to 1.3 V), giving rise to an anodic current (Chevion et al., 2000; Ligumsky et al., 2005). Each wave on the current reflects activity strength of several compounds sharing similar ability to donate an electron. The determined potential value on the x-axis (*Ep*) indicates presence of different types of LMWA (Fig. 2). The lower the oxidation potential, the higher the ability to donate electron(s) (Kohen et al., 2000; Ligumsky et al., 2005). The concentration of the reducing agents is determined by the value on the y-axis (*Ip*) and is measured as the area under a certain wave (*Ah*). Low and high *Ip/Ah* values indicate low or high concentrations of the tested compound(s), respectively (Ligumsky et al., 2005).

2.5. Statistical analysis

For all continuous parameters, the normality of data distribution was evaluated using the Shapiro-Wilk's test. Normally and non-normally distributed continuous parameters are reported as mean and SD or as median and range, respectively, and were compared between survivors and non-survivors using Student's *t*- or Mann-Whitney tests, respectively. Fisher's exact test was used to compare categorical variables between outcome groups. CV parameters were evaluated as predictors of outcome using the receiver operating characteristics (ROC) procedure. The optimal cutoff point was selected as the point that was associated with the least number of misclassifications. Spearman's rank or Pearson correlations were used to assess the correlation between continuous variables. Change in continuous parameters of the CV values over time was performed using the Friedman test. For all test applied $P \leq 0.05$ was considered statistically significant. All calculations were performed using statistical software ([e]SPSS 22.02 for Windows, Chicago, IL, USA).

3. Results

3.1. Signalment

The study included 30 dogs diagnosed with GDV, of which 16 and 14 were in the study and control groups, respectively. The following dog breeds were included in the study: mixed breed (5 dogs), German Shepherd (4 dogs), Labrador and Golden Retrievers, Cane Corso, Boxer and Shar-Pei (2 dogs each). All other breeds were represented by one dog each. There was no significant difference between the proportion of males and females (17/30 dogs, 56.6% vs. 13/30 dogs, 43.3%, respectively, $P = 0.67$). Median age of all dogs was 8.9 years (range 2.5–15 years), and median body weight was 35.0 kg (range 20–70 kg), with no significant difference between study groups ($P = 0.47$ and $P = 0.29$, respectively).

3.2. Lactate and gastric wall necrosis

Median serum lactate level upon admission was higher among non-survivors (7.2 mmol/l vs. 4.6 mmol/l, respectively), albeit insignificant ($P = 0.45$). Gastric wall necrosis (GWN) was visually suspected during surgery in 5/30 (16%) dogs, and was a significant risk factor (RF) for death (3/5, 60% vs. 1/25, 4%, respectively, $P = 0.009$).

3.3. Hospitalization and outcome

The overall mortality rate was 13% (4/30) with no significant difference between treatment and control groups (3 and 1, respectively, $P = 0.35$). None of the dogs were euthanized, and all died naturally after the surgery. Median hospitalization time period of all dogs was 24 h (range 12–82 h) with no significant difference between treatment groups ($P = 0.28$).

3.4. Cyclic voltammetry

Median *Ep* and *Ah* of 11 healthy control dogs were 0.35 Volt (range 0.30–0.39) and 2.89^{-7} μA (range 1.76^{-7}–6.11^{-7}), respectively. Median *Ep* at presentation and after 24 h in dogs with GDV were not significantly different from control dogs ($P = 0.092$ and $P = 0.18$, respectively). Median *Ah* at presentation was not significantly different from control dogs ($P = 0.13$), however it was significantly lower