Total antioxidant capacity in dogs with gastric dilatation and volvulus

I. Uhrikova, K. Rehakova, L. Rauserova-Lexmaulova, A. Lojek, J. Doubek

Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: The aim of this study was to determine total antioxidant capacity in dogs with gastric dilatation-volvulus syndrome (GDV) and its correlations with high mobility group box 1 protein (HMGB1) and lactate concentrations. Correlation analyses between the measured parameters and disease severity were also performed. Fourteen dogs with GDV and six control dogs were used in this study. Blood was collected at the time of admission and again in the early reperfusion period. To assess antioxidant capacity, total radical-trapping antioxidant parameter (TRAP) analysis was performed. No significant difference in TRAP values existed between healthy dogs and dogs with GDV at admission. In the reperfusion period, TRAP values decreased in six dogs and increased in eight dogs. Changes in TRAP values strongly correlated with HMGB1 values ($r = -0.83$, $P < 0.01$) in the reperfusion period. Strong correlations between disease severity and TRAP values, HMGB1 and lactate levels were also found.

Keywords: total radical-trapping antioxidant parameter (TRAP); high mobility group box 1 protein (HMGB1); gastric dilatation-volvulus syndrome (GDV); lactate

Gastric dilatation-volvulus syndrome (GDV) is an acute life-threatening disease in dogs; large breeds with deep chests are predisposed to the condition. Cranial movement of the pylorus from right to left is crucial for development of the disease and spontaneous repositioning becomes impossible due to the accumulation of gas in the stomach. Whether primary gas accumulation or gastric rotation occurs remains unclear (Monnet 2003). The spatial changes and distension of the stomach result in poor perfusion of the stomach and the proximal duodenum wall. Surgical intervention involving decompression and repositioning of the stomach is essential for successful treatment. Due to the pathogenesis of the disease and its treatment, GDV represents a model of ischaemia-reperfusion *in vivo*.

Reperfusion after a period of ischaemia is associated with the generation of reactive oxygen species (ROS), including oxygen free radicals, hydrogen peroxide, lipid peroxides and other molecules involved in tissue damage (Layton and Pazdernik 1993). Both the mucosa and the lumen produce oxygen free radicals in the gastrointestinal tract. In the intestine, the primary source of radicals is probably xanthine oxidase. Oxygen radicals are also produced by activated neutrophils with the formation of hydrogen peroxide and hypochlorite ions (Thomas and Balasubramanian 2004). The effect of ROS on tissues is inversely related to the tissue's antioxidant defensive capacity. Antioxidant capacity consists of endogenous antioxidants, which include hydrophobic molecules (α-tocopherol, β-carotene), aqueous antioxidants (ascorbic acid, bilirubin), and enzymes (catalase, glutathion peroxidasis) (Layton and Pazdernik 1993). Different methods for antioxidant capacity evaluation have been described, but there remains controversy regarding what the optimal method is (Walker et al. 2007). For determination of antioxidant status, we use the total radical-trapping antioxidant parameter (TRAP) which has been already used on experimental models of ischaemia-reperfusion in rats (Slavikova et al. 1998), and is similar to the method previously described in a study of antioxidant capacity in dogs with GDV (Walker et al. 2007).

Recently, high mobility group box 1 protein (HMGB1) was used as a marker of ischaemia-reper-

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fusion damage in dogs as well as humans (Tsung et al. 2005). HMGB1 is a nuclear protein that acts as a late pro-inflammatory cytokine but is also passively released during cell necrosis (Erlandsson and Andersson 2004). The connections between lactate production and gastric necrosis were described by de Papp et al. (1999).

The aim of this study was to determine the antioxidant capacity in dogs with GDV during periods of ischaemia and reperfusion, and the correlation of this capacity with HMGB1 levels, lactate concentrations and disease severity. Correlation analyses between the measured parameters were also performed.

MATERIAL AND METHODS

Animals

Fourteen dogs with GDV presented at the Small Animal Clinic of University of Veterinary and Pharmaceutical Sciences in Brno were included. Six females including one spayed individual and eight males including one castrated individual, with a mean weight of 43 kg and a mean age of six years, were used in this study. Routine medical and surgical treatment with gastropexy was provided to each dog according to individual needs. Six clinically healthy staff-owned dogs were used as the control group.

Blood sampling

Blood was collected pre-operatively (period of ischaemia) from the jugular vein into citrate-containing tubes, heparinised tubes and tubes that contained clotting activator. A second blood sampling was performed six to 10 h after surgical treatment (period of reperfusion). Tubes were centrifuged (15 min, 3000 rpm), and serum/plasma was stored at –20 °C until analysis.

Total radical-trapping antioxidant parameter (TRAP) analysis

A peroxyl radical reaction was observed by luminol-enhanced chemiluminiscence using a previously described method (Cizova et al. 2006). Briefly, the chemiluminiscence signal is driven by the production of luminol as derived radicals from the thermal decomposition of 2,2-azo-bis-2-amidinopropane hydrochloride (ABAP, Polyscience, Niles, IL, USA). The peroxyl radicals are scavenged by antioxidants, and the chemiluminiscence signal decreases. When the antioxidants are exhausted, chemiluminiscence signal recovers. The TRAP value is determined based on the period of time during which the plasma sample scavenges the peroxyl radicals. A known concentration (8.0 nmol) of trolox (Sigma-Aldrich, St. Louis, MO, USA), a water-soluble analogue of tocopherol, was used as a reference inhibitor instead of serum.

HMGB1 analysis

HMGB1 analysis was performed from the serum samples using an ELISA kit (IBL International GmbH, Hamburg, Germany) according to the manufacturer's instructions.

Lactate analysis

Lactate concentrations were measured on an automatised biochemical analyzer DPC Konelab 20i (Thermo Fisher Scientific, Finland) from heparinised plasma.

Scoring system

The severity of ischaemia-reperfusion injury depends on the period and grade of ischaemia. The time from the onset of clinical signs was used for quantification of the ischaemic period. The approximate degree of gastric rotation recorded by the surgeon (90°, 180°, 270°, 360°) was used for the determination of ischaemia grade. The disease severity score was obtained by multiplying the ischaemic period by the ischaemia grade.

Statistics

Data were evaluated using Microsoft Excel. Minimum, maximum, mean and median values of the TRAP, HMGB1 and lactate concentrations were reported in the control group, in dogs in the period of ischaemia ($TRAP_i$, $HMGB1_i$, $lactate_i$), and in the period of reperfusion ($TRAP_r$, $HMGB1_r$, $lactate_r$). The mean values are shown ± SD. The differences (Δ) in TRAP, HMGB1 and lactate during the periods of

ischaemia and reperfusion were calculated. Pearson's correlation coefficients were used for correlation analysis, and the Student's *t*-test was used to determine the significance of the differences between groups. Statistical significance was set at $P < 0.01$.

RESULTS

TRAP analysis

The mean TRAP value in healthy dogs was 290 ± 112 µmol/l. In the period of ischaemia, it was 273 ± 122 µmol/l, and in the period of reperfusion, it was 363 ± 307 µmol/l. No significant difference was observed between the TRAP values in healthy dogs and in dogs with GDV during the period of ischaemia ($TRAP_i$, $P = 0.77$) nor in reperfusion ($TRAP_r$, $P = 0.58$). Also, no significant difference was observed between $TRAP_i$ and $TRAP_r$ ($P = 0.32$). Minimal, maximal and median TRAP values are shown in Figure 1. $TRAP_r$ decreased in six dogs and increased in eight dogs.

HMGB1 values

A significant difference was apparent between HMGB1 values in the control group and in dogs with GDV in the period of ischaemia ($P < 0.01$). The mean HMGB1 value in the control group was 0.54 ± 0.65 ng/ml. In the period of ischaemia, it was 4.91 ± 4.17 ng/ml ($HMGB1_i$). In the period of reperfusion, it was 12.87 ± 20.62 ng/ml ($HMGB1_r$). Minimal, maximal and median HMGB1 values are shown in Figure 2. A strong significant correlation existed between ΔTRAP and $HMGB1_r$ values ($r = -0.82$, $P < 0.01$); other correlations are presented in Table 1.

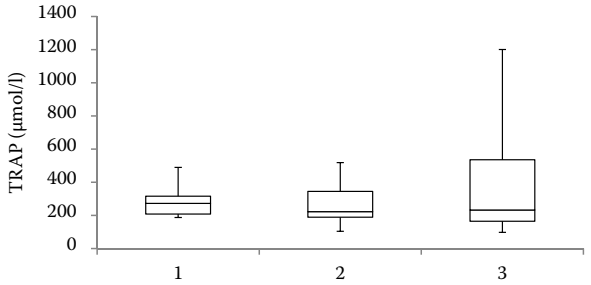

Figure 1. Total radical-trapping antioxidant parameter values

Bars denote the minimal and maximal values; boxes indicate 1st quartile, median, and 3rd quartile

1 = control group, 2 = period of ischaemia, 3 = period of reperfusion

Table 1. Total radical-trapping antioxidant parameter (TRAP) and high mobility group box 1 protein (HMGB1): *R*-values of significant Pearson's correlation coefficients

	$HMGB1_i$	$HMGB1_r$	ΔHMGB1
$TRAP_r$	0.80	0.83	–0.78
ΔTRAP	–0.73	–0.83	0.80

$TRAP_r$ = TRAP value after reperfusion, ΔTRAP = $TRAP_i$ (value during ischaemia) – $TRAP_r$, $HMGB1_i$ = HMGB1 value during ischaemia, $HMGB1_r$ = HMGB1 value after reperfusion, ΔHMGB1 = $HMGB1_i$ – $HMGB1_r$

Lactate concentrations

A significant difference was found in the lactate concentrations between the control group and the period of ischaemia in patients ($P < 0.01$). The mean lactate concentration was 3.35 ± 2.13 mmol/l in the period of ischaemia ($lactate_i$) and 3.27 ± 3.52 mmol/l in the period of reperfusion ($lactate_r$). In the control group, the mean lactate concentration was 1.45 ± 0.34 mmol/l. Minimal, maximal and median lactate values are shown in Figure 3. No significant correlation was found between the initial lactate levels and the TRAP or HMGB1 values. Lactate concentrations in the period of reperfusion ($lactate_r$) were strongly correlated with $HMGB1_r$ ($r = 0.93$, $P < 0.01$); other results are shown in Table 2.

Disease severity

Strong significant correlations between disease severity and $TRAP_r$ ($r = 0.81$, $P < 0.01$) or ΔTRAP ($r = -0.83$, $P < 0.01$), as well as between disease severity and $HMGB1_i$ ($r = 0.85$, $P < 0.01$), ΔHMGB1 ($r = -0.87$, $P < 0.01$), Δlactate ($r = -0.71$, $P < 0.01$) and $lactate_r$ ($r = 0.83$, $P < 0.01$), were found. The strongest correlation was between disease severity and $HMGB1_r$ ($r = 0.92$, $P < 0.01$).

Table 2. Lactate, total radical-trapping antioxidant parameter (TRAP) and high mobility group box 1 protein (HMGB1): *R*-values of significant Pearson's correlation coefficients

	$HMGB1_i$	$HMGB1_r$	ΔHMGB1	$TRAP_r$
$Lactate_r$	0.81	0.93	–0.89	0.82
ΔLactate	–0.74	–0.81	0.77	–0.75

$Lactate_r$ = lactate concentration after reperfusion, ΔLactate = $lactate_i$, (lactate concentration during ischaemia) – $lactate_r$, $HMGB1_i$ = HMGB1 value during ischaemia, $HMGB1_r$ = HMGB1 value after reperfusion, ΔHMGB1 = $HMGB1_i$ – $HMGB1_r$, $TRAP_r$ = TRAP value after reperfusion

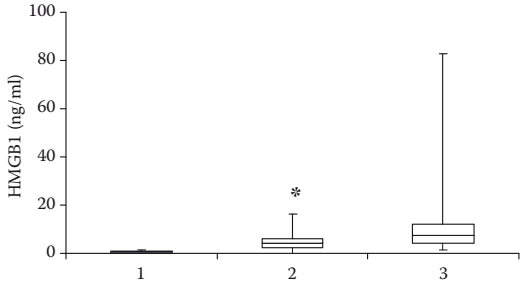

Figure 2. High mobility group box 1 concentrations in groups

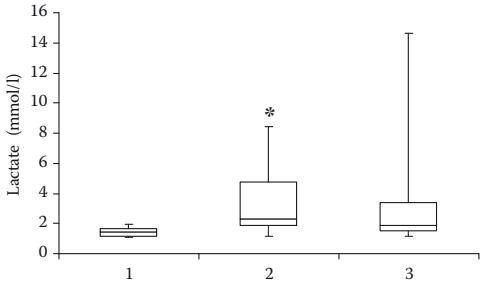

Figure 3. Lactate concentrations in groups

Bars denote the minimal and maximal values; boxes indicate 1st quartile, median, and 3rd quartile
1 = control group, 2 = period of ischaemia, 3 = period of reperfusion
*significant difference from control group

DISCUSSION

Few studies have focused on measuring antioxidant capacity during stomach or intestine ischaemia and reperfusion. Reports have demonstrated changes in TRAP values between control groups and the group of patients in the period of ischaemia and in the early (one to four hours) and late (one to four days) periods of reperfusion (Slavikova et al. 1998). Our results showed no significant difference in antioxidant capacity between control dogs and dogs with GDV during the period of ischaemia. Walker et al. (2007) described the same results in dogs with GDV by the method of oxygen radical absorbance capacity (ORAC, a method similar to TRAP). ORAC values in the period of ischaemia were considered to be normal based on the values of control dogs reported by Freeman et al. (2005). In contrast to other studies on GDV, an increase in TRAP values was observed during intestinal ischaemia in rats by Slavikova et al. (1998).

In experimental studies, TRAP values increased with the duration of the early period of reperfusion. It is believed that the increase in TRAP is associated with the release of antioxidants from their deposits (Slavikova et al. 1998). In our study no significant difference was found between the period of ischaemia (or control group) and the period of reperfusion, even though the extent of values was far wider in the period of reperfusion. An increase in $TRAP_r$ values was noted in eight dogs, and a decrease was observed in the remaining six patients. The reasons for these opposing changes remain unclear.

We did not analyse antioxidant capacity in the late period of reperfusion, but Walker et al. (2007) described a significant decrease in ORAC after one day, and the same tendency was observed by Slavikova et al. (1998) after two days in the period of reperfusion.

Our results show the elevation of HMGB1 in patients with GDV. The strong correlation between $HMGB1_r$ and ΔTRAP probably reflects the release of HMGB1 from necrotic cells due to ischaemia-reperfusion cell damage. These results are in accordance with previous findings by Tsung et al. (2005), who reported the up-regulation of HMGB1 expression in the liver after ischaemia-reperfusion injury in mice. In contrast to our study, they were unable to detect HMGB1 in the serum. We cannot exclude false-positive HMGB1 levels due to cell death during surgical treatment, as we did not have a sham-operation group in the study. Ishida et al. (2011) observed elevated HMGB1 levels after ovario-hysterectomy in healthy beagle dogs with the highest levels occurring at 72 h after surgery. A slight elevation in HMGB1 levels was also observed during the first 24 h, but this part of study was limited by the low number of patients. In addition, we demonstrated an elevation of HMGB1 levels, as well as their correlation with ischemia-reperfusion injury evaluated by TRAP measurement and disease severity.

Lactate is a product of anaerobic glycolysis during tissue hypoxia, and elevated levels are associated with the impairment of its removal. Lactate concentration and kinetics have been described as a prognostic indicator as well as a marker of gastric necrosis in patients with GDV (de Papp et al. 1999; Zacher et al. 2010). A poor outcome was observed when lactate concentrations at admission were higher than 6 mmol/l (de Papp et al. 1999), while Zacher et al. (2010) proposed an initial cut-off value of 9 mmol/l. Two dogs that died in our study had lactate levels of 8.4 mmol/l and 5.66 mmol/l. Previously, we described the correlation between $lactate_i$ concentration and $HMGB1_i$ values (Uhrikova et al. 2011). In this study, only a mild correlation was found (r = 0.50) between these two measurements.

That may be due to the relatively small number of animals in the group, because we proved a strong correlation between $HMGB1_i$ values and lactate concentrations in dogs with GDV (n = 25) in a long-term study (r = 0.75, unpublished data). Moreover, several differences in lactate and HMGB1 kinetics exists that may result in this poor initial correlation. The sensitivity of a lactate cut-off of 6 mmol/l for gastric necrosis was only 61%, and the specificity was 88% in a previous study (de Papp et al. 1999). Based on this data, we performed a correlation analysis of lactate concentrations with TRAP and HMGB1. No correlation between the initial lactate concentration and any TRAP or HMGB1 parameter was found, but results indicated a strong correlation of both lactate levels (r = 0.82) and HMGB1 (r = 0.83) with antioxidant status in the reperfusion period. Despite this, $HMGB1_r$ strongly correlates with disease severity, though our scoring system could be affected by subjective rating. In patients with GDV, lactate as well as oxygen radicals may be produced by other hypoxic tissues due to shock, and peripheral blood used for analysis may be affected by overall body status. Experimental studies that involve measuring ROS, TRAP, HMGB1 and lactate in blood collected simultaneously from the portal vein and peripheral veins are needed.

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Corresponding Author:

Ivana Uhrikova, University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Medicine, Palackeho 1–3, 612 42 Brno, Czech Republic

Tel. +420 541 562 228, E-mail: uhrikovai@gmail.com