Chapter 12
Superoxide Dismutase and Glutathione Peroxidase in Dogs with Leishmaniasis Following Antimoniate Therapy

D. Britti, S. Sconza, G. Massimini, C. Civitella, A. Luciani, and L. Lavecchia

Abstract The effect of antimony treatment on antioxidative enzyme activities of erythrocyte superoxide dismutase (SOD) and blood glutathione peroxidase (GSHPx) and the associations between these enzyme activities and hematological indices of anemia (pre- and posttreatment) was studied in dogs with leishmaniasis. Twelve dogs with leishmaniasis before and after 60 days of therapy with *N*-methylglucamine antimoniate were used. No significant differences in antioxidant SOD and GSHPx activities or hematological indices, including HGB level, RBC number, HCT, MCV, MCH, and MCHC, were detected between pre- and post-antimony treatment. Results indicated that the presence or absence of previous treatment with antimony had no statistically identifiable effects on antioxidant enzyme activity or hematological indices.

Keywords Anemia • Canine visceral leishmaniasis • GSHPx • SOD • Therapy

Abbreviations

CanVL	Canine visceral leishmaniasis
GSHPx	Glutathione peroxidase
HCT	Hematocrit
HGB	Hemoglobin

D. Britti (✉) • L. Lavecchia
Dipartimento Medicina Sperimentale e Clinica, Università degli Studi di Catanzaro "Magna Græcia", Catanzaro, Italy
e-mail: britti@unicz.it

S. Sconza • C. Civitella • A. Luciani
Dipartimento Scienze Cliniche Veterinarie, University of Teramo, Teramo, Italy

G. Massimini
Medico Veterinario Libero Professionista, Ortona, Chieti, Italy

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MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
RBCs	Red blood cells
ROS	Reactive oxygen species
Sb^v	Pentavalent antimony
SOD	Superoxide dismutase

12.1 Introduction

Reactive oxygen species (ROS) contribute to the development and persistence of anemia during canine visceral leishmaniasis (CanVL) through multiple mechanisms (Weed and Reed 1966; Sen et al. 2001). The interaction between ROS and hemoglobin (HGB) results in the denaturation and precipitation of protein with the production of methemoglobin (Biswas et al. 1997), influencing the average lifespan of red blood cells (RBCs) (Weiss 1982). The peroxidation of RBC membrane lipids induced by ROS leads to the alteration of cell-membrane morphology and plasticity, promoting premature erythrocatheresis (Biswas et al. 1997). Among blood enzymes with free-radical scavenging activity, the erythrocyte form of superoxide dismutase (SOD) and the erythrocyte and plasmatic forms of glutathione peroxidase (GSHPx) are of particular interest (Guemouri et al. 1991). In our previous study, we detected a significant increase in the mean value of erythrocyte SOD activity and a significant reduction of RBCs and HGB in dogs with CanVL as compared with healthy dogs (Britti et al. 2008). In that study, the mean value of GSHPx activity in dogs with CanVL was decreased as compared with that of healthy dogs, even though this difference was not significant. The aim of this study was to evaluate the effect of Sb^v treatment on erythrocyte SOD and blood GSHPx activities in dogs with CanVL and to determine the potential associations between these enzyme activities and RBCs, HGB, and others indices of anemia before and after treatment.

12.2 Materials and Methods

Twelve dogs (7 males) of several breeds, average age 5.2 ± 2.0 years, with spontaneous CanVL as detected by serological (IFAT) and parasitological (lymph node cytology) methods, and with negative serological test results for ehrlichiosis and babesiosis, were enrolled in the study. Data were obtained at the time of diagnosis of CanVL (T_0) and after 60 days of therapy (T_{60}) with 75 mg/kg of *N*-methylglucamine antimoniate, SC q12h equivalent to 21.38 mg/kg of Sb^v. Twenty-five clinically healthy dogs (10 males) of several breeds, average age 4.1 ± 2.5, with no diseases as judged by the methods listed above, were used as controls (CTRL). From each of the 37 dogs, a 6.0-mL blood sample was drawn by

jugular venopuncture and divided into an EDTA tube for CBC analysis (Advia120®) and a LiHe tube for the measurement of antioxidative enzyme activities. Particularly, SOD activity was measured after isolation and lysis of RBCs, while GSHPx activity was determined in whole blood. Tests were performed using a clinical chemistry analyzer (Olympus-AU400®), according to the respective manufacturer's specification (Ransod® and Ransel®, RandoxLab).

Shapiro–Wilk tests indicated that the data followed a normal distribution. The Student's *t*-tests were used to evaluate the differences in the mean values of antioxidative SOD and GSHPx activities and hematological indices, including HGB, RBCs, HCT, MCV, MCH, and MCHC between T_0 and T_{60} groups (paired data) and between T_0 or T_{60} and CTRL groups (unpaired data). Linear regression analysis was used to evaluate the potential associations between these variables (pre- and posttreatment). All calculations were performed using a commercial statistical package (GraphPad Prism®).

12.3 Results

Means ± SDs, 95% confidence intervals ($CIs_{95\%}$) of the means, and *P* values for testing the differences between groups are reported in Tables 12.1 and 12.2. The comparison between T_0 and CTRL groups was reported in our previous study (Britti et al. 2008). In the current study, the comparison between T_0 and T_{60} groups revealed no significant differences in the mean values for SOD and GSHPx activities and the hematological indices, including HGB, RBCs, HCT, MCV, MCH, and MCHC levels. For example, no significant differences were detected between pre- and posttreatment with Sb^v for each of the considered variables in this study (Table 12.1). The comparison between T_{60} and CTRL groups revealed a mean level of SOD activity significantly higher ($P < 0.05$) at T_{60} and mean levels of HGB, RBCs, HCT, MCV, and MCH significantly lower ($P < 0.05$) at T_{60}. No significant differences were detected for GSHPx and MCHC levels (Table 12.2). Simple linear regression analyses demonstrated significant negative associations among the variables at T_{60}, similar to those detected at T_0 in our previous study

Table 12.1 Hematological indices and activities of antioxidative enzymes in dogs with CanVL before (T_0) and after (T_{60}) Sb^v therapy

	T_0 Mean ± SD ($C.I._{95\%}$)	T_{60} Mean ± SD ($C.I._{95\%}$)	$P < 0.05$
RBC (x106/μL)	5.64 ± 0.83 (5.11–6.17)	5.49 ± 0.8 (4.98–6.0)	n.s.
HGB (g/dL)	12.42 ± 2.04 (11.12–13.71)	12.46 ± 2.0 (11.19–13.73)	n.s.
HCT (%)	35.67 ± 6.01 (31.86–39.49)	35.43 ± 5.41 (32.0–38.87)	n.s.
MCV (fL)	60.99 ± 9.2 (55.15–66.84)	64.58 ± 3.4 (62.42–66.75)	n.s.
MCH (pg)	21.69 ± 2.24 (20.27–23.11)	22.72 ± 1.8 (21.58–23.86)	n.s.
MCHC (g/dL)	33.78 ± 4.44 (30.95–36.6)	35.15 ± 1.57 (34.15–36.15)	n.s.
SOD (U/gHGB)	114.2 ± 18.0 (102.7–125.6)	115.2 ± 21.6 (101.5–129.0)	n.s.
GSHPx (U/L)	988.7 ± 222.3 (847.5–1130.0)	872.1 ± 271.9 (699.3–1044.8)	n.s.

Table 12.2 Hematological indices and activities of antioxidative enzymes in dogs with CanVL after Sb^v therapy (T_{60}) and in healthy dogs (CTRL)

	T_{60} Mean ± SD (C.I.$_{95\%}$)	CTRL Mean ± SD (C.I.$_{95\%}$)	$P < 0.05$
RBC (x106/μL)	5.49 ± 0.8 (4.98–6.0)	6.94 ± 0.61 (6.68–7.2)	<0.0001
HGB (g/dL)	12.46 ± 2.0 (11.19–13.73)	16.86 ± 1.67 (16.16–17.57)	<0.0001
HCT (%)	35.43 ± 5.41 (32.0–38.87)	47.65 ± 4.55 (45.72–49.57)	<0.0001
MCV (fL)	64.58 ± 3.4 (62.42–66.75)	68.58 ± 2.78 (67.41–69.76)	<0.001
MCH (pg)	22.72 ± 1.8 (21.58–23.86)	24.3 ± 1.28 (23.76–24.84)	<0.01
MCHC (g/dL)	35.15 ± 1.57 (34.15–36.15)	35.43 ± 2.22 (34.49–36.37)	n.s.
SOD (U/gHGB)	115.2 ± 21.6 (101.5–129.0)	82.6 ± 9.0 (78.8–86.4)	<0.0001
GSHPx (U/L)	872.1 ± 271.9 (699.3–1044.8)	1025.3 ± 284.6 (905.2–1145.5)	n.s.

(Britti et al. 2008). Particularly, simple linear regression analysis indicated a strong negative linear associations between SOD activity and HGB concentration ($R^2 = 0.96$; $P < 0.0001$), SOD activity and RBCs ($R^2 = 0.76$; $P < 0.0001$), and SOD activity and HCT ($R^2 = 0.90$; $P < 0.0001$) at T_{60}. No significant associations were detected at T_{60} between SOD and GSHPx activities and between GSHPx activities and hematological indices. Additionally, a minimal negative association ($R^2 = 0.38$; $P < 0.05$) was detected between SOD activities at T_0 and HGB concentrations at T_{60}, and a minimal positive association ($R^2 = 0.39$; $P < 0.05$) was detected between GSHPx activities at T_0 and HGB concentrations at T_{60}. Finally, a multiple linear regression analysis was used to develop a moderately accurate algorithm ($R^2 = 0.50$; $P < 0.05$) to predict HGB concentrations at T_{60} by measuring SOD and GSHPx activities at T_0. The relationship between these variables (in the absence of colinearity between SOD and GSHPx at T_0) is described by the following formula: HGB_{T60} (g/dL) = 13.901 + 0.004 × $GSHPx_{T0}$ (U/L) − 0.045 × SOD_{T0} (U/gHGB). In the developed algorithm, parameters of regression indicate a positive association between $GSHPx_{T0}$ activities and HGB_{T60} concentrations and a negative association between SOD_{T0} activities and HGB_{T60} concentrations.

12.4 Discussion

Results of our study revealed that the presence or absence of a previous treatment with Sb^v had no statistically identifiable effect on antioxidative enzyme activities and hematological indices. No significant differences in SOD and GSHPx activities and hematological indices were detected in dogs with CanVL before and after therapy with Sb^v (T_0 and T_{60}). Erythrocyte SOD activities were significantly higher and HGB, RBCs, HCT, MCV, and MCH levels were significantly lower in dogs with CanVL at 60 days after therapy as compared to clinically healthy dogs (Figs. 12.1 and 12.2). On the basis of these findings, we assume that these parameters are significantly altered in dogs with CanVL as compared with those of control dogs, regardless of the Sb^v treatment. This result suggests that in dogs

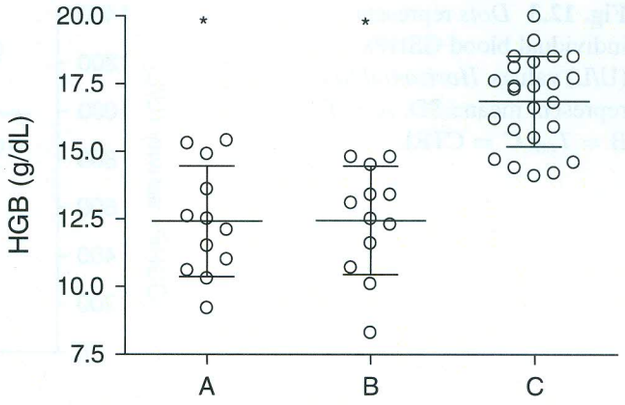

Fig. 12.1 *Dots* represent individual HGB (g/dL) values. *Horizontal lines* represent mean ± SD. A = T_0; B = T_{60}; C = CTRL. *$P < 0.05$ as compared to CTRL

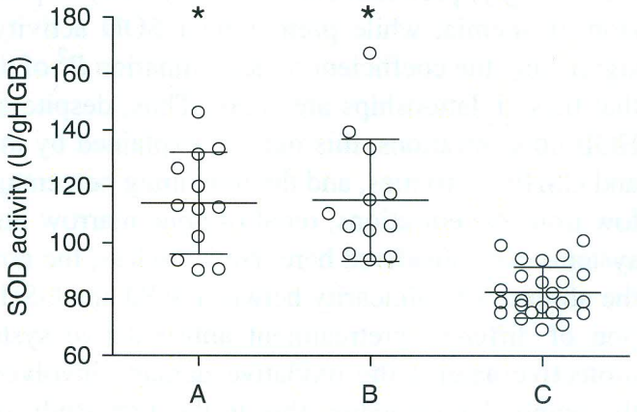

Fig. 12.2 *Dots* represent individual erythrocyte SOD (U/gHGB) values. *Horizontal lines* represent mean ± SD. A = T_0; B = T_{60}; C = CTRL. *$P < 0.05$ as compared to CTRL

with CanVL, Sb^v therapy does not improve the redox state or the underlying anemia. This may be explained, at least in part, by other antioxidative systems not evaluated in this study, by the degree of renal impairment or failure, which is not always detectable through routine tests (Ciaramella et al. 1997), or by the fact that the Sb^v therapy alone may not be sufficient to determine the improvement of the hematological indices and redox states in dogs with CanVL (Erel et al. 1999). For example, in hamsters with experimental visceral leishmaniasis, the combination of antioxidants, such as α-tocopherol, ascorbic acid, and flavonoids, with the Sb^v therapy resulted in a greater reduction of ROS and parasitemia (Sen et al. 2005), and a greater increase in HGB concentrations and RBC half-lives as compared to the Sb^v therapy alone (Sen et al. 2004). It is important to note that while the levels of SOD activity and hematological indices in dogs with CanVL were similar before and after therapy with Sb^v, but significantly different as compared to those of control dogs, the levels of GSHPx activities before therapy were similar to that of control dogs, and were basically reduced after Sb^v therapy (Fig. 12.3). As previously reported during chronic experimental infections in hamsters (Sen et al. 2001) and cutaneous leishmaniasis in humans (Kocyigit et al. 2003), this finding, although without statistical significance, may suggest a depletion of the GSHPx antioxidative system during chronic CanVL infections in dogs. However, the lack of a statistical significance for this finding underlines the need for increased sample size.

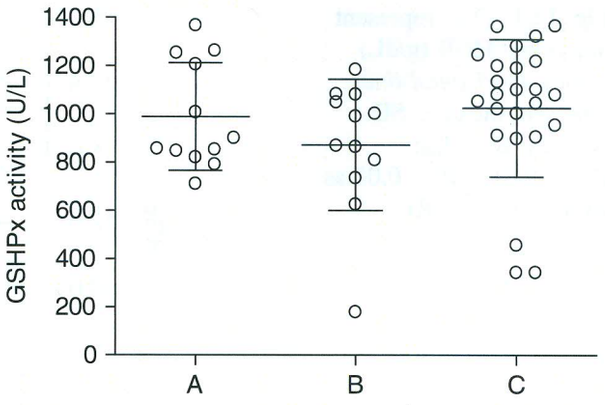

Fig. 12.3 *Dots* represent individual blood GSHPx (U/L) values. *Horizontal lines* represent mean±SD. A = T_0; B = T_{60}; C = CTRL

Interestingly, pretreatment GSHPx activity is positively related with the progression of anemia, while pretreatment SOD activity is inversely related. Although significant, the coefficient of determination R^2 of the developed algorithm indicates that these relationships are weak. Thus, despite a 50% variance in posttreatment HGB concentrations, this may be explained by the variance of pretreatment SOD and GSHPx activities, and the remaining percentage is due to other factors, such as low iron concentrations, renal or bone marrow impairment, or other antioxidative systems not considered here. Nevertheless, the normal distribution of the data and the absence of colinearity between SOD and GSHPx activities support the evaluation of different pretreatment antioxidative systems that may be predictive or protective against the oxidative damage involved in anemia during CanVL. To the authors' knowledge, this is the first study on the comparison of SOD and GSHPx activities in dogs with CanVL before and after therapy with Sb^v. As previously observed during CanVL (Bildik et al. 2004), RBCs are exposed to an oxidative stress that could be responsible for their premature removal from circulation. Our results suggest that this damage persists even after Sb^v therapy. A study is in progress to examine the efficacy of the combination of antioxidants with the Sb^v therapy in dogs with CanVL.

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