THE EFFECT OF ENDOGENOUSLY PRODUCED CARBON MONOXIDE ON THE OXYGEN STATUS OF DOGS INFECTED WITH *BABESIA CANIS*

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ABSTRACT

Carboxyhaemoglobin fractions were found to be significantly higher ($P<0,05$) in dogs (n=5) with severe babesiosis than in control subjects (n=5). The enzymatic conversion of haem to biliverdin by haem oxygenase is the only known source of endogenous carbon monoxide. We propose that the increased production of endogenous carbon monoxide following the haemolysis associated with babesiosis, results in the carboxyhaemoglobinaemia observed in this study. The superimposition of carboxyhaemoglobinaemia on severe anaemia results in further compromise of the oxygen status of dogs with severe babesiosis, and probably plays a role in the pathogenesis of the hypoxic tissue damage associated with this condition.

Key words: Canine, babesiosis, *Babesia canis*, carbon monoxide, carboxyhaemoglobin

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INTRODUCTION

Babesia canis infection of dogs often results in tissue hypoxia which increases anaerobic tissue metabolism, production of metabolic acids, shock and death[1]. The transport of oxygen from the lungs to the tissues depends on haemoglobin function. Maegraith et al. observed a left shift of the oxygen dissociation curve (ODC), and hypoxic effects that were similimar to those associated with canine babesiosis, in normal dogs following subcutaneous injection of sodium nitrate[2]. These authors were, however, unable to detect methaemoglobin in the blood of dogs infected with *B. canis*. They therefore speculated that the hypoxic tissue damage that occurs with canine babesiosis was due to local factors within the tissues themselves[2].

Following haemolysis, haem is metabolised, iron is salvaged and biliverdin formed. This reaction is catalysed by haem oxygenase. The enzyme specifically catalyses the cleavage of the α-methene bridge. This pathway requires molecular oxygen and produces carbon monoxide (CO) as a metabolite[7]. The α-methene carbon is the only known endogenous source of CO[7]. The affinity of CO for the iron of haem is 218 times greater than that of oxygen[6]. It competes with O_2 for binding sites on the haem, thus lowering the effective haemoglobin concentration of the blood. Binding of CO also influences the haemoglobin molecule to such an extent that the remaining binding sites have an increased affinity for oxygen. This, however, decreases the ability of haemoglobin to off-load oxygen at the tissues[5].

The advent of accurate multiwavelength oximeters for in vitro measurement of oxygen saturation, haemoglobin concentration, and carboxy- and methaemoglobin fractions has resulted in the development of a number of new parameters to describe the oxygen status of patients[5,6]. The oxygen extraction tension (P_x) is one such parameter[5]. P_x is defined as the oxygen tension required to extract 2,3 mmol of oxygen per litre of blood (at constant pH and pCO_2). This parameter integrates the effects of changes in arterial pO_2, oxygen capacity, and oxyhaemoglobin affinity on the delivery of O_2 to the tissues[5]. At present, P_x appears to be the most relevant parameter for evaluating the overall arterial oxygen status[3].

The purpose of the present investigation was to compare the carboxy- and methaemoglobin concentrations of blood from dogs with *B. canis* infections with that of clinically healthy dogs. Furthermore, the theoretical effects of increased carboxyhaemoglobin concentrations on the oxygen status of dogs with clinical babesiosis were investigated.

MATERIALS AND METHODS

Anaerobic, heparinised blood samples were collected aseptically from the cephalic veins of control dogs (n=5) and dogs with natural *B. canis* infections (n=5). The control group comprised 5 clinically healthy dogs. The 5 principal subjects were all presented at the Outpatients Clinic of the Faculty of Veterinary Science, University of Pretoria. Clinical examination revealed that all subjects were pyrexic and examination of a stained blood smear (CAMS Quick stain, C.A. Milsch (Pty) Ltd) showed the presence of *B. canis* parasites. Four of the 5 subjects were also severely anaemic. Due to the severity of the condition in all of the subjects, they were admitted for further investigation. Blood samples were collected prior to the administration of any therapeutic agents.

The total haemoglobin concentration

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(ctHb), carboxyhaemoglobin fraction (FCOHb) and methaemoglobin fraction (FMetHb) of the blood samples were measured using a semi-automated haemoximeter (OSM3, Radiometer A/S, Copenhagen). The instrument was calibrated using the standard calibration procedures and standard solutions (Hemoximetry-Qualicheck, Radiometer A/S, Copenhagen) were processed prior to, and following analysis of samples.

The oxygen status algorithm[6] was used to calculate estimates of the oxygen extraction tension (P_x) for a healthy dog, an anaemic dog and a dog with anaemic and concurrent carboxyhaemoglobinaemia. The means of the FCOHb and FMetHb of blood from control and principal subjects were compared using an analysis of variance procedure in SAS[4]. Significance was set at $P<0,05$.

Table1: **Total haemoglobin concentration, carboxyhaemoglobin and met-haemoglobin fractions of blood from healthy dogs and dogs with severe clinical babesiosis**

	Control (n=5)			*Babesia canis* infected (n=5)		
	Mean	SD	Range	Mean	SD	Range
ctHb (g ℓ^{-1})	159,4	18,5	133,0-185,0	70,8	54,7	39,0-168,0
FCOHb (%)	1,1*	0,2	0,8-1,4	3,0*	1,3	1,6-5,2
FMetHb (%)	0,0	0,0	0,0-0,0	0,8	0,4	0,5-1,0

SD - Standard deviation
ctHb - total haemoglobin concentration
FCOHb - Carboxyhaemoglobin fraction
FMetHb - Methaemoglobin fraction
*- Signifies that the means are significantly different ($P<0,05$)

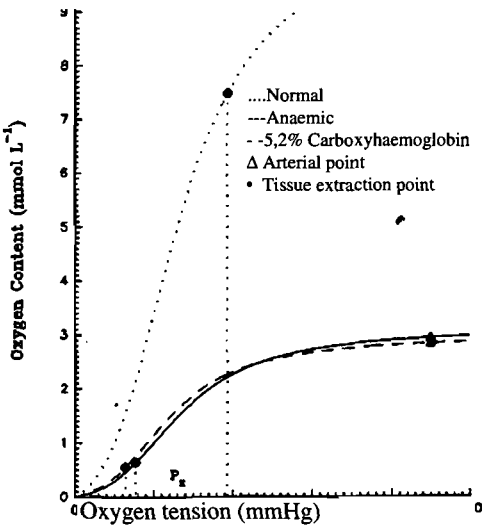

Fig. 1: **Derived blood oxygen binding curves of blood from a normal dog, from an anaemic dog and from a dog with anaemia and concurrent carboxyhaemoglobinaemia. The arterial blood oxygen tension was taken to be 90,0 mmHg in all cases.**

RESULTS

The haemoximetry data are summarised in Table 1. The mean of the FCOHb of blood from the dogs with clinical babesiosis was significantly greater than that of the control group of dogs. The means of FMetHb did not differ significantly between the 2 groups. The oxygen binding curves of blood derived for a dog with a ctHb of 160 g ℓ^{-1} and a FCOHb of 1,1%, for a dog with a ctHb of 47 g ℓ^{-1} and a FCOHb of 1,1% and for a dog with a ctHb of 47 g ℓ^{-1} and a FCOHb of 5,2% are depicted in Fig. 1. The estimated oxygen extraction tensions (P_x) of the blood from the normal dog, the anaemic dog and the dog with anaemia and concurrent carboxyhaemoglobinaemia were 38,6, 15,4 and 12,7 mm Hg, respectively.

DISCUSSION

This study revealed that there was a significant increase in the carboxyhaemoglobin fraction of blood obtained from dogs with severe babesiosis. This increased FCOHb was associated with a marked anaemia in 4 of the 5 principal subjects (mean ctHb of 47 g ℓ^{-1}). The mean methaemoglobin fractions of blood from control dogs and babesiosis cases were not significantly different. This finding supports that of a previous study in dogs[2]. In man, a P_x of less than 33,75 mmHg is considered to be indicative of tissue hypoxia[5]. The P_x value calculated for a dog with a ctHb of 47 g ℓ^{-1} was 15,4 mmHg. This value was approximately 40% that of a normal dog with a ctHb of 160 g ℓ^{-1}. The superimposition of a FCOHb of 5,2% upon a ctHb of 47 g ℓ^{-1} resulted in a P_x of 12,7 mmHg. This implied that the oxygen status of the dog with concurrent carboxyhaemoglobinaemia and anaemia was approximately 20% worse than that of a dog with anaemia alone. The metabolic salvaging of iron from haem is viewed as an essential homeostatic mechanism[7]. This pathway uses O_2 and produces endogenous CO as a metabolite. Normally the CO produced by this reaction is slowly excreted via the respiratory tract and is of little physiological consequence. Following severe haemolysis, however, the amount of CO produced by haem oxygenase may have physiologically significant effects. These effects include a marked shift to the left of the ODC and a reduced effective haemoglobin concentration, resulting in further compromise of the oxygen status of the already anaemic animal. The increased FCOHb with concurrent reduced haemoglobin concentration, is analogous to a superimposition of CO toxicity upon severe anaemia.

We conclude that the increased FCOHb observed in severe cases of canine babesiosis may be important in the pathogenesis of the severe tissue hypoxia often associated with this condition. In the light of these findings, further study of the oxygen status of the blood in less severe cases of canine babesiosis, and in babesiosis of other domestic animal species should be undertaken. Our findings also suggest that therapy of severe babesiosis should be aimed at radical improvement of the oxygen status of the patient. This could include blood transfusion with cross-matched blood, thus limiting further potentially disastrous oxidation of haem, oxygen therapy and measures to shift the ODC to the right. The effect of such threapeutic measures could be quantitated using the recently developed parameters describing the oxygen status of blood.

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REFERENCES

1. Button C 1976 Fluid therapy in canine babesiosis. Journal of the South African Veterinary Association 47: 285-287
2. Maegraith B, Gilles H M, Devakul K 1957 Pathological process in *Babesia canis* infections. Zeitschrift für Tropenmedizin und Parasitologie 8: 485-514
3. Ringsted C, Eliasen K, Gothgen I H, Siggaard-Andersen O 1990 Positive correlation between the arterial oxygen extraction tension' and mixed venous pO_2 but lack of correlation between 'the oxygen compensation factor and cardiac output in 38 patients. Scandinavian Journal of Clinical and Laboratory Investigation 203 Supplement 50: 67-73
4. SAS Institute Inc. 1988 SAS/STAT User's Guide, Release 6.03 Edition SAS Institute Inc., Cary, North Carolina
5. Siggaard-Andersen O, Gothgen I H, Wimberley P D, Fogh-Andersen N 1990 The oxygen status of the arterial blood revised: relevant oxygen parameters for monitoring the arterial oxygen availability. Scandinavian Journal of Clinical and Laboratory Investigation 203 Supplement 50: 17-28
6. Siggaard-Andersen O, Siggaard-Andersen M 1990 The oxygen status algorithm: a computer program for calculating and displaying pH and blood gas data. Scandinavian Journal of Clinical and Laboratory Investigation 203 Supplement 50: 29-45
7. Wells M S, Awad W M Jr. 1986 Iron and heme metabolism. In: Devlin T M (ed.) Textbook of Biochemistry with Clinical Correlations 2nd edn John Wiley & Sons, New York: 855-874