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SHORT COMMUNICATION

Two methods to adapt the human haemoglobin–oxygen dissociation algorithm to the blood of white rhinoceros (*Ceratotherium simum*) and to determine the accuracy of pulse oximetry

Agnes Haymerle, Felix Knauer & Chris Walzer
Research Institute of Wildlife Ecology, Veterinary University Vienna, Vienna, Austria

Correspondence: Agnes Haymerle, Research Institute of Wildlife Ecology, Savoyenstrasse 1, A-1160 Vienna, Austria. E-mail: agneshaymerle@hotmail.com

Abstract

Objectives To adapt the algorithm for the calculation of oxygen saturation to the blood characteristics of the white rhinoceros by two different methods and to determine the accuracy of conventional pulse oximetry measurements.

Study design Adaptation of two mathematical models of the oxygen dissociation curve (ODC).

Animals Twenty-five captive white rhinoceros (*Ceratotherium simum*), including 12 males and 13 females, aged 6–32 years.

Methods During 33 anaesthetic events, 94 arterial blood gas samples with 72 simultaneous pulse oximetry measurements were analysed. The calculation of oxygen saturation was adapted to the characteristics of rhinoceros blood using two different methods. Firstly, a mathematical model developed in 1984 and, secondly, an oxygen status algorithm (OSA) produced by the same developer in 2005 were tested for their applicability for clinical use.

Results When arterial partial pressure of oxygen is >7.98 kPa (60 mmHg), oxygen saturation exceeds 95%. At partial pressures of 6.12–6.52 kPa (46–49 mmHg) Method 1 determined oxygen saturations of 92.5–95.3% and Method 2 oxygen saturations of 90.2–91.6%. Both methods resulted in similar ODCs and accounted for the low p50 value of rhinoceros blood. Method 1 provided better adaptation in respect to the physiological parameters of the rhinoceros, especially with regard to the Bohr effect, than Method 2. Pulse oximetry was an unreliable method of monitoring arterial oxygen saturation during general anaesthesia in this species.

Conclusion Adapting the oxygen saturation algorithm to consider the left shift of the ODC provides a useful tool for monitoring oxygen status, especially as pulse oximetry is insufficiently accurate. Experimental determination of the complete Hill curve is required to further validate and optimize the algorithm for use in the white rhinoceros.

Clinical relevance The method will facilitate the accurate interpretation of oxygen saturation calculated by blood gas analysis in white rhinoceros.

Keywords blood gas, *Ceratotherium simum*, haemoglobin saturation, oxygen dissociation curve, pulse oximetry.

Introduction

Achieving general anaesthesia in large quadrupeds, such as the white rhinoceros (*Ceratotherium simum*

simum and cottoni), is a challenging task for veterinarians but is indispensable in conservation management and medical interventions. Close monitoring of arterial oxygen saturation by blood gas analysis and pulse oximetry during anaesthesia is essential as hypercapnia and, especially, hypoxemia are reported during general anaesthesia in white rhinoceros. Blood gas analysis and pulse oximetry are two methods of ensuring the adequate monitoring of oxygen status in the arterial blood. Its continuous monitoring, low cost and simple application makes pulse oximetry very popular, whereas blood gas analysis is more costly and is discontinuous. Both methods have one big disadvantage: they are validated for use in humans or, in the best case, in domestic animals, but not in wildlife. Today, pulse oximetry shows good accuracy in pets such as dogs (Burns et al. 2006) and farm animals. Pulse oximetry devices generally do not overestimate oxygen saturation significantly (Matthews et al. 2003; Burns et al. 2006). A pulse oximeter estimates oxygen saturation (SpO_2) based on light absorbance of defined wavelengths of human oxy- and deoxyhaemoglobin. Variant human haemoglobins are responsible for low SpO_2 measurements (Verhovsek et al. 2010). Measuring the arterial pressure of oxygen (PaO_2), the unbound oxygen content in the blood, with a blood gas analyser is, by contrast, species-independent. Generally SpO_2 and oxygen saturation by the blood gas analyser (SaO_2) should correlate closely. In the majority of animals the standard measurements are satisfactory, but in species with extreme p50 values (partial pressure at which haemoglobin is 50% saturated with oxygen), such as the white rhinoceros (Baumann et al. 1984), or extreme adaptations to physiological changes in pH or 2,3-diphosphoglycerate (2,3-DPG), such as in camels, this may not be the case. The algorithm for calculating oxygen saturation in blood gas machines requires haemoglobin-specific validation. Humans show a p50 of about 3.99 kPa (30 mmHg), whereas the p50 at a pH of 7.4 and temperature of 37 °C in rhinoceros is extremely low at 2.66 kPa (20 mmHg). With decreasing pH, oxygen binding affinity decreases more than in humans because of the strong Bohr effect of −0.62, whereas oxygen affinity changes little with 2,3-DPG and adenosine triphosphate (ATP) (Baumann et al. 1984). The reason for this special characteristic of rhinoceros haemoglobin may reflect a specific glutamic acid residue at position β2 in haemoglobin (Mazur et al. 1982). However, other than in Ball et al. (2011), this fact has rarely been considered in evaluations of the oxygen status of the anaesthetized white rhinoceros.

The algorithm to calculate oxygen saturation (sO_2) can be adapted to calculate species-specific sO_2 values, if data on the p50, Bohr effect, effect of 2,3-DPG and ATP (Mazur et al. 1982; Baumann et al. 1984) and physiological blood chemistry (Citino & Bush 2007) are available, as they are for the white rhinoceros. Herein, we present two methods to adapt the algorithm for species-specific validation by empirical fitting throughout the entire Hill curve.

Materials and methods

Captive white rhinoceros (*C. s. simum and cottoni*) of both sexes at reproductive age were anaesthetized during the years 1999–2003 within the European Endangered Species Programme (EEP) for serial clinical reproductive monitoring to elucidate the causes of female and male reproductive failure. All procedures were conducted according to the guidelines of the ethics committee of the University of Veterinary Medicine Vienna and the respective legislation in the countries in which the procedures were performed. Clinical examinations of the reproductive organs included transrectal ultrasonography, electroejaculation and artificial inseminations under general anaesthesia. A sternal or lateral recumbent state was achieved with a combination of detomidine–HCL (Domosedan; Orion Corp., Finland), butorphanol (Torbugesic; Fort Dodge Animal Health, IA, USA) and etorphine–acepromazine (Large Animal Immobilon; C-Vet Veterinary Products, UK).

Heart rate (HR; beats minute^{-1}), arterial oxygen saturation (SpO_2; %) by pulse oximeter placed at the ear after skin scraping (Nellcor NP-20; Tyco Healthcare Group LP, Nellcor Puritan Bennett Group, CA, USA), and respiratory frequency (f_R, breaths minute^{-1}) were recorded. Within the frame of routine anaesthetic monitoring, heparinized anaerobic blood samples were collected from an auricular artery and immediately processed with a portable blood gas analyser (i-Stat; SDI Sensor Devices, WI, USA). During 33 anaesthetic events in 25 individual rhinoceros, 94 blood samples were taken and analysed [mean ± standard (SD) deviation: 2.85 ± 1.30 samples per procedure; 12 males with a mean ± SD age of 20.0 ± 7.2 years (range: 6–32 years); 13 females with a mean ± SD age of 25.6 ± 7.2 years (range: 8–31 years)]. Two

animals were sampled twice during individual events and three animals were sampled three times during individual events.

To calculate arterial oxygen saturation from the results of blood gas analysis, we used two different methods: firstly, we adapted the mathematical model of the haemoglobin–oxygen curve (Siggaard-Andersen et al. 1984, 1988), and, secondly, we used the oxygen status algorithm (OSA) (version of September 18, 2005) provided by O. Siggaard-Andersen (http://www.siggaard-andersen.dk).

Method 1

The first method involved the adaptation of the algorithm for the oxygen dissociation curve (ODC) after Siggaard-Andersen et al. (1984, 1988).

A mathematical model giving oxygen saturation as a function of PaO_2 was described by Siggaard-Andersen et al. in 1984 and extended in 1988. Parameters of human blood were replaced by the blood characteristics and physiology of the white rhinoceros: p50 of 2.66 kPa (20 mmHg); Bohr effect of −0.62 (Baumann et al. 1984); physiologic $PaCO_2$ of 6.52 kPa (49 mmHg), and physiologic body temperature of 36.8 °C (Citino & Bush 2007).

We determined x_s of the point of symmetry (which is described by x_s and y_s) by nested intervals:

$x = \ln(P_aO_2\ [\text{kPa}]) \rightarrow x_{50} = 0.973$ [p50 of 2.66 kPa PaO_2 (Baumann et al. 1984)]

$y = \ln(SO_2/(1 - SO_2)) \rightarrow y_s = 1.875 \rightarrow y_{50} =$ 0 [value of p50, Baumann et al. 1984)]

$n_0 = 2.87 + a^*k$

$h = 3.3 + a$

$k = (n_0 - 1)/h = 0.5343.$

Under standard conditions of pH 7.4 and 37 °C, a and b are expected to be zero [equation 5 in Siggaard-Andersen et al. (1984)].

$x_s = y_s - y + x + h^*\tanh[k^*(x - x_0)]$ (Siggaard-Andersen et al. 1984, 1988)

$x_s = 1.6460.$

Oxygen saturation was calculated by the ODC function represented by the following equations:

$sO_2 = e^y/(1 + e^y)$

$y = 1.875 + x - x_0 + h\ ^*\tanh[k^*(x - x_0)]$

$x_0 = x_s + a + b$

$a = cB^*(\text{pH} - 7.4) + 0.09^*\ln(PaCO_2/PpCO_2) - 0.368^*x\text{HbCO} - 0.174^*x\text{Hi}$

$cB = (-1)^*\ln(10)^*\beta$ [$\beta = -0.62$, Bohr effect (Baumann et al. 1984)]

$PaCO_2 = 6.517$ kPa [physiologic $PaCO_2$ of white rhinoceros (Citino & Bush 2007)]

$x\text{HbCO} = 0.005$ [substance fraction of carboxyhaemoglobin, after Siggaard-Andersen et al. (1988)]

$x\text{Hi} = 0.005$ [substance fraction of haemoglobin, after Siggaard-Andersen et al. (1988)]

$b = 0.055^*[T/(273.15–310.15)]$ [T = 36.8 °C, physiologic body temperature of white rhinoceros (Citino & Bush 2007)].

As 2,3-DPG has little influence on oxygen affinity (Baumann et al. 1984) and no fetal haemoglobin is expected in the blood of rhinoceros at reproductive age, we removed these parameters from the model.

Method 2

Method 2 was based on the OSA provided by O. Siggaard-Andersen (version of September 18, 2005). The OSA program opens a data page with default values for the underlying algorithm. The input fields can be selected. In order to estimate SaO_2 using the OSA, we considered as constant the following parameters: blood temperature of 37 °C; concentration of total haemoglobin (ctHb) of 12.9 g dL^{-1}; ambient pressure (Pamb) of 101 kPa (760 mmHg), and oxygen content of inspired air of 21% (FO2I). The measured blood gas values PaO_2, $PaCO_2$ and pH were inserted into the input fields. The known rhino Bohr effect could not be integrated in Method 2. In this version it is not possible to change the p50 value and therefore we manually iterated the p50 by changing the SaO_2. When the p50 value was 2.66 kPa (20 mmHg), we took the SaO_2 value. The results of this manual iteration process are provided in Table S1. Because of its large body mass, body temperature in the captive rhinoceros during anaesthesia remains constant at 37 °C (C. Walzer, personal data, 2011).

Results

The adapted algorithms for oxygen saturation take into account the low p50 of rhino haemoglobin and therefore lead to a left shift of the ODC compared with the algorithm used by the commercial blood gas analyser (Fig. 1). Oxygen saturation calculated by Method 1 is on average 16.38% higher, and by Method 2 14.12% higher than the values derived from the commercial human-adapted blood gas analyser [analysis of variance (ANOVA): Methods 1 and 2, $p < 0.001$]. Methods 1 and 2 demonstrate that oxygen saturation is actually higher than 95% when PaO_2 is higher than 7.98 kPa (60 mmHg).

The simultaneously recorded SpO_2 values from the pulse oximeter are far below the actual level of oxygen saturation. It is striking that the variation in SpO_2 values is large throughout the entire recorded PaO_2 range.

Discussion

The underestimation of oxygen saturation by blood gas analysis and pulse oximetry originates from two different methodological factors. Firstly, the blood gas analyser does not consider species-specific shifts of the ODC and the pulse oximeter measures the light absorption at two fixed wavelengths. The calculation by Method 1 allows the inclusion of the important physiological parameters, including the p50 value, the Bohr effect (Baumann et al. 1984), and the physiological $PaCO_2$, pH and body temperature (Citino & Bush 2007) of white rhinoceros. The mathematical model of Siggaard-Andersen et al. (1984) is very flexible. Here, the model was adapted by shifting the point of symmetry referring to the low p50 value. The Hill slope was not changed, but the shoulder of the Hill curve was flattened to avoid overestimation by reducing the *h* parameter to 3.0 instead of 3.5 (Siggaard-Andersen et al. 1984). Using Method 2, the OSA, we discovered that entering the blood gas values into the OSA program is relatively simple but the iterative approach to account for the low p50 is not practical. Method 2 is notable for two limiting factors: neither the Bohr effect nor the physiological $PaCO_2$ of rhino blood can be considered. Of minor impact is the model's use of the effects of 2,3-DPG and ATP of human blood, neither of which can be removed, although both factors are known to have little effect in rhinoceros blood (Baumann et al. 1984). A convenient tool in the OSA program refers to the provision of the total physiological *v–a* shunt fraction. Cardiac output per square metre/body mass is higher in rhinoceros than the calculated output from the OSA program. It contains negative physiological shunt fractions, although negative physiological shunting is impossible. This indicates that the rhino has a higher cardiac output than the human, expressed per square metre of body surface (O. Siggaard-Anderson, personal communication, 2010). During general anaesthesia, information concerning the total physiological *v–a* shunt fraction may represent a valuable parameter for monitoring, but should be regarded as a relative value. However, both methods provide similar SaO_2 values and highlight the importance of adapting the commercial algorithms to the rhinoceros in order to correctly monitor oxygenation status during anaesthesia. The large methodological underestimation of oxygen saturation measured with the pulse oximeter is clearly apparent when plotting SpO_2 against SaO_2 calculated by Method 1

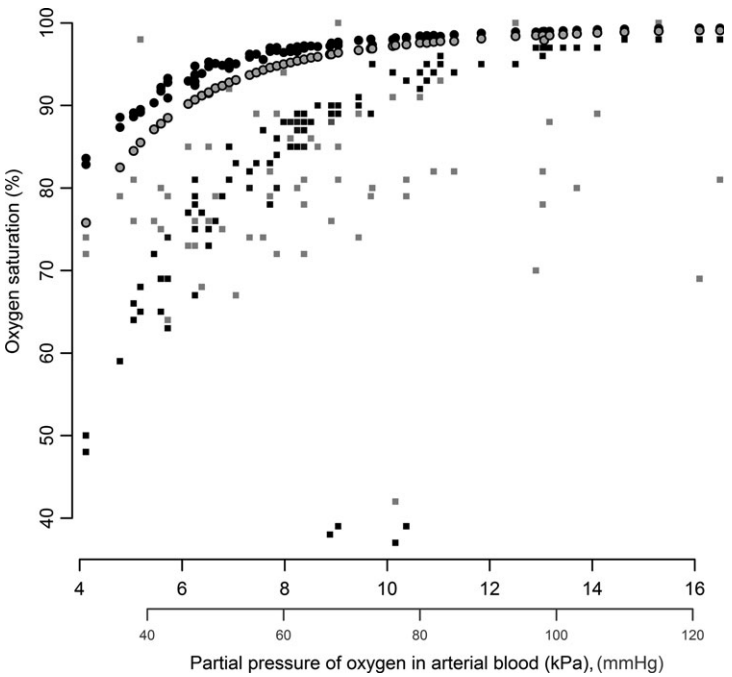

Figure 1 The oxygen dissociation curve for white rhinoceros with differently calculated oxygen saturations (sO_2) from the measured partial pressures of oxygen in arterial blood during general anaesthesia: sO_2 values calculated by the blood gas analyser (■), by Method 1(●), by Method 2 (●) and measured simultaneously by pulse oximeter (■).

(Fig. S1). This may be explained by several factors: the vasoconstrictive nature of the employed α_2-agonist may lead to a reduced blood flow in the periphery or the probe clip of the pulse oximeter may lead to a reduced blood flow in the sampled tissue held in the clip. Furthermore, rhinoceros haemoglobin may have different peak wavelength absorbance for oxy- and deoxy-haemoglobin. In humans, variant haemoglobin leads to low SpO_2 measurements (Verhovsek et al. 2010). Accurate and responsible monitoring of rhinoceros anaesthesia with the pulse oximeter requires the determination of the wavelengths of maximal absorbance for oxy- and deoxy-haemoglobin and the subsequent adaptation of devices. In addition, the complete Hill curve must be determined to further validate and optimize the algorithm for the species-specific ODC in blood gas devices.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Arterial blood gases values measured in white rhinoceros with oxygen saturations calculated by a portable blood gas analyser, a pulse oximeter and by two different mathematical methods.

Figure S1. The underestimation of oxygen saturation measured with the pulse oximeter (SpO2) is clearly apparent when plotting SpO_2 against the oxygen saturation (sO_2) calculated by Method 1.

