Anesthesiology
68:111–114, 1988

Accuracy of Pulse Oximetry During Arterial Oxyhemoglobin Desaturation in Dogs

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An animal model was developed to evaluate the accuracy of pulse oximetry over a wide range of oxyhemoglobin desaturation. The fractional inspired oxygen concentration was varied from 0.03–1.0 in five anesthetized dogs. One hundred and twelve simultaneous pulse oximeter oxygen saturation measurements (SpO_2) and IL 282 CO-Oximeter arterial oxygen saturation (SaO_2) measurements were made. Variance of SpO_2 was increased for SaO_2 < 22%. Linear regression analysis of the data for SaO_2 > 22% produced the equation $y = 0.93x + 9.8$ ($r^2 = 0.97$). The mean difference between SpO_2 and SaO_2 was +5.5% ± 4.2% (SD) over the range of 22–100%. Spectral analyses of oxygenated (O_2Hb) and reduced (RHb) canine and human hemoglobins were performed. The absorption spectra of canine O_2Hb and RHb were nearly identical to those of human O_2Hb and RHb. Therefore, 1) SpO_2 measurements in dogs at SaO_2 > 22% are relatively accurate, and 2) hemoglobin absorption characteristics support the contention that such canine pulse oximeter studies can be extrapolated to humans. (Key words: Blood, hypoxia, dog: hemoglobin; oxyhemoglobin saturation. Measurement techniques: pulse oximetry; spectrophotometry. Monitoring: pulse oximetry.)

THE ROLE OF PULSE oximetry as a monitor of arterial oxygen saturation (SaO_2) has been steadily expanding. Recently suggested applications include its use in situations where moderate to severe arterial oxyhemoglobin desaturation may occur: in the operating room,[1–5] the recovery room,[6,7] the delivery room,[8,9] and the intensive care unit.[10–13] Furthermore, the instrument may also be potentially useful for the continuous monitoring of oxygenation in animals during experimental conditions; both for models of hypoxia, and also for the *in vivo* evaluation of newly developed clinical monitoring equipment.[14]

The accuracy of pulse oximetry at steady state SaO_2 < 70% is unknown. Previous investigations validating the methodology during normal and moderately depressed SaO_2 have been performed with human subjects,[10–12,15–21] but reproducible and controlled experimentation in humans during severe hypoxemia is unethical. Animal experimentation using pulse oximetry, on the other hand, has not been widely used. Measurements made by pulse oximeters in animals may be valid, however, since absorption by hemoglobin in the visible spectrum (400–700 nanometers) is similar among all vertebrates,[22] and at least one of the wavelengths of light used in two-wavelength pulse oximetry falls in the visible spectrum.[23] We were, therefore, stimulated to develop an *in vivo* canine model of hypoxemia to determine the accuracy of pulse oximetry, especially during severe arterial oxyhemoglobin desaturation. To determine whether the results of our canine experiments could be extrapolated to humans, an *in vitro* analysis of the absorption characteristics of oxygenated (O_2Hb) and reduced (RHb) canine hemoglobin was undertaken and compared to adult human hemoglobin at both visible and infrared wavelengths (those used in pulse oximetry).

Materials and Methods

In Vivo Study

Five mongrel, adult male dogs (15–25 kg) were anesthetized with pentobarbital and orotracheally intubated. A femoral venous catheter was placed for intravenous infusions, and a femoral arterial catheter was inserted both to measure blood pressure and to obtain arterial blood gas specimens. Rectal temperatures of 36.5–37.5° C were maintained by covering the dogs with plastic bags and using heating blankets and warming lamps. A pulse oximeter sensor (Nellcor®, D-25) was sewn across the tongue and connected to a pulse oximeter (Nellcor®, N-100). Arterial blood pressure, heart rate, end-tidal P_{CO_2}, and the pulse oximeter's pulse wave pattern and oxygen saturation (SpO_2) were continuously recorded on a Gould-Brush® 8-channel recorder. After paralysis with pancuronium, positive pressure ventilation was initiated with a Harvard ventilator using a respiratory rate of 40/min and a tidal volume of 5–7 ml/kg. This formula for minute ventilation was chosen to minimize the respiratory variation in the pulse oximeter pulse wave tracing and to maintain a normal Pa_{CO_2}. Blood pressure was maintained near baseline, with an epinephrine infusion as required during severe oxyhemoglobin desaturation.

During the experiment, FI_{O_2} was varied from 0.03–1.0 using a mixture of oxygen and nitrogen. At each level of FI_{O_2}, after a 2-min period of SpO_2 stabili-

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Received from the Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins School of Medicine, Baltimore, Maryland. Accepted for publication August 11, 1987. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Las Vegas, Nevada, October, 1986.
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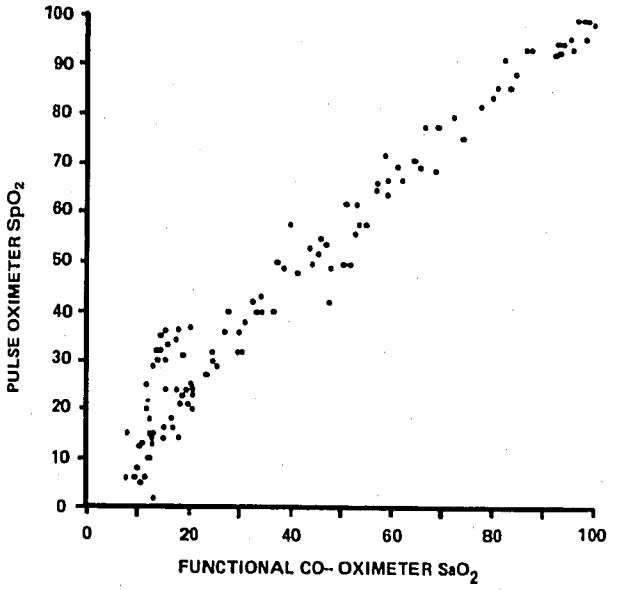

FIG. 1. Simultaneous measurements (n = 112) of pulse oximeter SaO_2 (ordinate) *versus* functional Co-Oximeter SaO_2 (abscissa), as measured in dogs. Regression analysis yields the line $y = 0.97x + 6.93$, $r^2 = 0.99$.

zation, heparinized arterial blood specimens were obtained for analysis. All specimens were collected anaerobically and placed on ice. Pa_{O_2}, Pa_{CO_2}, and *p*H were measured on a blood gas machine (Radiometer America, Inc., ABL_3). A CO-Oximeter (Instrumentation Laboratory Inc., IL 282), calibrated for canine blood, measured % methemoglobin (MetHb), % carboxyhemoglobin (COHb), and % oxyhemoglobin (O_2Hb). For the purpose of comparison with SpO_2,[21] we calculated functional SaO_2 from the CO-Oximeter fractional measurements using a standard formula:

$$\% \text{ functional } SaO_2 = \frac{\% \ O_2Hb}{100 - (\% \text{ MetHb} + \% \text{ COHb})} \times 100$$

There were 17–25 paired measurements of SaO_2 and SpO_2 in each animal.

All data were pooled for analysis, and linear regression statistics were used to compare the calculated functional CO-Oximeter SaO_2 to the simultaneous SpO_2 (obtained from the strip chart recordings). The mean difference (bias) between pulse oximeter SpO_2 and functional CO-Oximeter SaO_2 was calculated, as well as the standard deviation of the difference (precision).

In Vitro Study

Fresh, heparinized blood samples were obtained from a dog and an adult human, and centrifuged to remove the plasma and white blood cells. The red blood cells were washed three times in normal saline. A volume of 2.5 ml of packed red blood cells was suspended in 4 ml of normal saline. The hemoglobin concentration of this red cell suspension was determined by a CO-Oximeter (Instrumentation Laboratory Inc., IL 282). A 2% solution of octylphenoxypolyethoxyethanol in saline (diluent used to lyse the red blood cells) was combined with the red cell suspension in a 1:4 ratio, and the resulting mixture was gently agitated, and then centrifuged at 10,000 RPM, 4° C for 30 min. The supernatant (containing free hemoglobin) was collected, and oxygenated for 10 min prior to spectral analysis.

A scanning Fourier transform spectrophotometer (Bomem®, DA3.02) was used for the spectral analysis. Samples were analyzed in a 1-cm pathlength cuvette. The optical density was measured at wavelengths of 600–1050 nanometers, and the millimolar extinction coefficients were calculated. Oxygenated diluent was used as a reference for the oxygenated hemoglobin solution. After analysis of both oxygen saturated solutions, analysis of the deoxygenated solutions was performed. To deoxygenate the solutions, 15 mg of sodium dithionite was added to both the diluent and the hemoglobin solutions immediately before spectral analysis. The cuvettes were covered with parafilm and analyzed in a nitrogen-purged chamber. The deoxygenated diluent was used as a reference for the deoxygenated hemoglobin solution. The absorption spectra for canine O_2Hb and RHb were compared to those of human O_2Hb and RHb, specifically at the wavelengths of light used by the Nellcor pulse oximeter.

Results

In Vivo Study

There were 112 simultaneous SpO_2 and SaO_2 measurements made over a functional SaO_2 range of 8–100%. When SpO_2 is compared to the functional CO-Oximeter SaO_2 (fig. 1), there is greater variance in SpO_2 for SaO_2 values less than 22%. Therefore, we analyzed the SaO_2 range < 22% separately from SaO_2 > 22%. Linear regression analysis for SaO_2 > 22% yields the equation $y = 0.93x + 9.8$ ($r^2 = .97$), with a standard error of the y estimate of 3.9. The 95% confidence interval of the slope is ±.04. For SaO_2 < 22%, $y = 1.43x - 0.4$ ($r^2 = .29$), with a standard error of the y estimate of 8.1. The 95% confidence interval of the slope is ±.68. The bias between SpO_2 and functional CO-Oximeter SaO_2 over the range of 22–100% is +5.5% ± 4.2% (SD, precision), and over the SaO_2 range of 8–22% is +5.9% ± 8.1%.

In Vitro Study

The absorption spectra for canine and human O_2Hb and RHb are shown in figure 2. At the wavelengths of light employed by the pulse oximeter (660 nm, nominal, and 920 nm, nominal§), the millimolar extinction coefficients of canine O_2Hb and RHb are nearly identical to those of human O_2Hb and RHb (table 1).

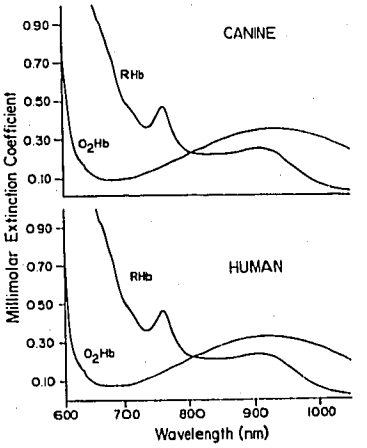

FIG. 2. Absorption spectra (λ/ϵ curve) for canine and human oxygenated (O_2Hb) and reduced (RHb) hemoglobin.

Discussion

The ability to accurately measure low levels of arterial oxygen saturation may be of importance in various clinical settings. For example, in newborns in the intensive care unit, SpO_2 of less than 10% may be present.[24] Also, in the delivery room[8,9] and pediatric[11,12] and adult[13] intensive care units, significant oxyhemoglobin desaturation has been detected using pulse oximetry. Since pulse oximeters are becoming extensively used in these settings, the validity of measurements obtained during moderate to severe arterial oxyhemoglobin desaturation must be verified.

In our *in vivo* canine experiment, SpO_2 closely reflected functional CO-Oximeter SaO_2 in the range of 22–100%. Over this range, there is an average bias of +5.5% (pulse oximeter reading higher than the functional CO-Oximeter SaO_2). Although there is greater variance in SpO_2 at $SaO_2 < 22\%$, the highest SpO_2 measured in this range was 37%. Therefore, even at $SaO_2 < 22\%$, SpO_2 measurements are sensitive indicators of hypoxemia.

For the purpose of this study, functional CO-Oximeter SaO_2 (IL 282) was used as the standard for comparison with SpO_2. The accuracy of the IL 282 CO-Oximeter at all levels of SaO_2 allows such comparison to be made. The CO-Oximeter's accuracy has been demonstrated against a direct measure of arterial oxygen content (Lexington Instruments Corp., Lex O_2 Con-TI.).[25]

To our knowledge, the absorption spectra of canine hemoglobin over the range of 600–1050 nanometers have never been previously published. The spectral analysis of canine hemoglobin yielded absorption spectra that are virtually identical to those previously published for human O_2Hb and RHb. Our hypothesis is that it is predominantly the heme moiety that absorbs light at these wavelengths, and, although the globin chains are different in dogs and humans, the prosthetic group of heme, ferroprotoporphyrin IX, is common to all vertebrates.[26] Therefore, we would expect that any mammalian hemoglobin containing normal heme (divalent iron atom, oxygen, and four porphyrin rings) would have similar absorption characteristics at these wavelengths. Because absorption at two wavelengths within this spectrum (660 nm, nominal, and 920 nm, nominal), are the basis for most clinical pulse oximetry measurements,[21,23] one could also conclude that the validity of experimental results obtained using pulse oximetry in any mammal can be extrapolated to other mammals, including humans.

All currently available two-wavelength pulse oximeters measure absorbence at wavelengths within the same spectra, and all include an application of Beer's law in their analyses.[23] However, various algorithms are used by different manufacturers in the analysis of input data. Although we have evaluated the accuracy of one model of pulse oximeter in the present study, other brands or models may be more or less accurate in this or any other given measurement setting, depending upon how well their algorithms apply.

In conclusion, measurements made using pulse oximetry during severe arterial oxyhemoglobin desaturation in dogs appear to be relatively accurate. There is reason to believe, based on the similarity of hemoglobin absorption characteristics at the wavelengths used in pulse oximetry, that such measurements would also be valid in humans.

TABLE 1. Millimolar Extinction Coefficients (ϵ)* for Human and Canine Hemoglobins at 660- and 920-nm Wavelengths

	Human (ϵ)	Canine (ϵ)
O_2Hb		
660 nm	0.08	0.08
920 nm	0.33	0.31
RHb		
660 nm	0.92	0.87
920 nm	0.23	0.21

§ User's Manual for the Nellcor Pulse Oximeter Model N-100C; Nellcor Inc, Hayward, CA, Catalog No. A2044, Rev. A.

* Optical density of an absorbing substance in a concentration of 1 mmole/l measured with a path length of 1 cm.

The authors wish to thank Donald D. Duncan, Ph.D., and Michael E. Thomas, Ph.D., of the Applied Physics Laboratory of The Johns Hopkins University for their assistance in the spectral analysis of hemoglobin.

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