Porcine-specific hemoglobin saturation measurements

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Departments of 1Anesthesiology and 4Anatomy, Physiology and Genetics, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814; 2Walter Reed Army Institute of Research, Washington, District of Columbia 20307; and 3Department of Physiology, University of Munich, Munich, Germany 80539

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Serianni, Richard, Jed Barash, Timothy Bentley, Pushpa Sharma, John L. Fontana, Darin Via, Jochen Duhm, Rolf Bunger, and Paul D. Mongan. Porcine-specific hemoglobin saturation measurements. J Appl Physiol 94: 561–566, 2003. First published October 18, 2002; 10.1152/japplphysiol.00710.2002.—The determination of O2 consumption by using arteriovenous O2 content differences is dependent on accurate oxyhemoglobin saturation measurements. Because swine are a common experimental species, we describe the validation of CO-oximeter for porcine-specific oxyhemoglobin saturation. After developing a nonlinear mathematical model of the porcine oxyhemoglobin saturation curve, we made 366 porcine oxyhemoglobin saturation determinations with a calibrated blood-gas analyzer and a porcine-specific CO-oximeter. There was a high degree of correlation with a calibrated blood-gas analyzer and a porcine-specific CO-oximeter. There was a high degree of correlation with minimal variability (r2 = 0.99, SE of the estimate = 5.2%) between the mathematical model and the porcine-specific CO-oximeter measurements. Bland-Altman comparison showed that the CO-oximeter measurements were biased slightly lower (−0.4 vol%), and the limits of agreement (±2 SD) were 0.7 and −1.5 vol%. This is in contrast to a 10–20 vol% error if human-specific methods were used. The results show excellent agreement between the nonlinear model and CO-oximeter for porcine-specific oxyhemoglobin saturation measurements. In contrast, comparison of the porcine-specific oxyhemoglobin saturations with saturations obtained by using human methods highlights the necessity of species-specific measurement methodology.

CO-oximetry; oxyhemoglobin; mathematical modeling

EXPERIMENTAL WORK IN HEMODILUTION, systemic hypoxia, hemorrhage, and ischemia-reperfusion routinely produces low levels of oxygenation and extremely low hemoglobin saturations. To obtain accurate data on global and regional O2 delivery and consumption during these conditions, the ability to measure or calculate species-specific oxyhemoglobin (HbO2) saturation is required. Because of the known differences between human hemoglobin and that of other animal species used for scientific study, companies have developed CO-oximeter coefficient sets specific for some laboratory animals. As with other mammals, porcine hemoglobin has a significant sequence difference from human hemoglobin (5–7, 19). These sequence differences alter the functional properties of hemoglobin and change the electrostatic interactions that modulate the O2 affinity (24). Subsequently, there is a difference in the HbO2 saturation characteristics of porcine and human blood as demonstrated by the decreased O2 affinity of porcine hemoglobin and the resulting increased 50% saturation with molecular oxygen (P50) (2, 4, 11, 15, 16, 22, 25). As with dogs and rats, this hemoglobin sequence difference results in differences in absorbance coefficients for the hemoglobin species at the specific CO-oximeter wavelengths. Thus relying on a human or other animal coefficient sets for the determination of HbO2 saturation will result in data that are not accurate. Although species-specific CO-oximeters are available for humans, dogs, and rats, the validation of an accurate porcine-specific CO-oximeter for laboratory use is currently lacking. To resolve this deficiency, we compiled laboratory hemoglobin saturation data that had previously only been used in classic Hill plots to graphically estimate the P50 of porcine hemoglobin (10, 15). These data were used to generate a descriptive mathematical model of the complete porcine HbO2 dissociation curve. In addition, using fresh porcine blood samples, Instrumentation Laboratories (IL) independently generated a porcine-specific coefficient set for direct measurement of HbO2 saturation using the IL 682 CO-oximeter. Subsequently, using fresh porcine blood obtained from instrumented anesthetized swine, we determined the HbO2 saturation determined by using the porcine-specific CO-oximeter and the HbO2 saturation calculated by using the porcine mathematical model. The agreement of these independent methods of porcine HbO2 saturation was compared with the HbO2 saturation determined by using a human-specific HbO2 saturation methods. These studies highlight the potential for significant systematic error when incor-
MATERIALS AND METHODS

Mathematical modeling of the porcine and human HbO2 dissociation curve. Porcine HbO2 saturation data generated by polarographic methods were combined with those generated by spectrophotometric methods for the generation of a mathematical model describing the full porcine HbO2 saturation curve. We compiled a database with a total of 213 porcine HbO2 saturation data points from 19 separate experimental series: 141 data points came from young adult porcine blood with normal 2,3-diphosphoglycerate levels in which hemoglobin saturation was measured by spectrophotometric methods (15), and 71 of the HbO2 saturation data points were generated polarographically (4). Although the HbO2 saturation data were measured differently, the experimental methods in terms of incubation and equilibration with O2, PCO2, pH, and temperature (37°C) were identical. Thus the data were combined to mathematically model the complete O2 dissociation curve. Furthermore, there were 34 HbO2 values from 11 experimental series determined spectrophotometrically and 18 values determined polarographically from eight experimental series at the critical hypoxic range (P02 = 0–25 Torr). Each of the 19 experimental series was assigned one zero-saturation value at an assumed P02 = 0. The database also contained 6 values for P02 ≥ 98 Torr (4).

Because O2 saturation of hemoglobin as a function of P02 is sigmoidal and can be described as a nonlinear rectangular hyperbola with cooperativity, we used a general nonlinear rectangular hyperbola mathematical model with an exponentiated variable to account for the sigmoidal nature of the Hb-O2 saturation (S) relationship (8). This model yielded an equation in the following general form

\[
\frac{S}{100} = \left( \frac{S \cdot P_{02}}{S \cdot P_{02}^n + b} \right) \cdot \frac{\beta}{\beta + b}
\]

Because our goal was only to develop a highly descriptive model of the porcine HbO2 saturation curve and not to validate the previous work that described the P50 and the molecular binding sites for O2, we chose to use the simpler form in Eq. 1, instead of the classic Hill equation in which \( b \) would be exponentiated (\( \beta^n \)) and \( b \) would define the P50. Using the maximum likelihood routines of Gauss 3.5 and Gaussx 3.7 (Aptech Systems, Kent, WA), we fitted the sigmoidal nonlinear rectangular hyperbola equation to the porcine data as previously described (14). At the extremes, the limits of saturation were assumed 0 and 100%. Furthermore, because pH and temperature can influence HbO2 binding, correction factors were added to the model (12, 21, 23, 24, 26). The correction for pH (\( \Delta \rho H \)) was based on the Bohr effect where \( [\Delta \log(P02) / \Delta \rho H] = -0.3 \) and \(-0.48\) for swine and humans, respectively, and \( \Delta \rho H = (7.4 - \text{measured pH}) (3, 23) \). Thus a “pH factor” of \(-0.3 \cdot \Delta \rho H \) or \(-0.48 \cdot \Delta \rho H \) was added to the models to correct the measured PO2. Temperature correction in the model was accomplished according to Severinghaus (23) by using \( \Delta \rho H = 0.031 \) (37°C – measured temperature) for human data and Willford and Hill (26) by using \( \Delta \rho H = 0.022 \) (37°C – measured temperature) for the swine data. This temperature correction of pH is subsequently translated into a change in PO2 by use of the Bohr effect.

The mathematical model of the human HbO2 dissociation curve was derived from 135 single or mean observations relating PO2 and hemoglobin saturation in human blood at 37°C and pH 7.4 (2, 4, 9, 16, 18, 23).

IL 682 CO-oximeter coefficient determination for porcine blood. Heparinized whole blood was obtained from Yorkshire swine (30–35 kg) and shipped overnight on ice to IL, where it was prepared for the determination of the porcine coefficient set for the IL 682 CO-oximeter. The animal coefficient set is an inverse \( 4 \times 4 \) matrix of the relative absorption coefficients for the porcine blood at the IL 682 measuring wavelengths (535, 585, 594, and 626 nm). In brief, IL personnel used standard validated laboratory procedures (on file at IL) to produce and measure porcine blood samples that consisted of 100% HbO2, 100% deoxyhemoglobin, 100% methemoglobin, and 100% carboxymethemoglobin. From these samples, IL determined the coefficient set for swine, which was subsequently programmed into the IL 682 CO-oximeter.

Construction of in vitro porcine HbO2 dissociation curve. Gas tanks of 100% N2, O2, and CO2 were connected to a Cameron Instruments (CIC) gas-mixing flowmeter that delivered the water-saturated gases in desired proportions to a CIC dual equilibrator (Cameron Instruments, Brownsville, TX). Six Yorkshire swine were used on separate days for these experiments. In preparation, they were anesthetized with halothane, intubated, and ventilated to maintain the arterial P02 at 40 Torr. After percutaneous placement of a 20-gauge femoral arterial cannula, 3-ml fresh heparinized blood samples were obtained and immediately added to each of the spinning chambers of the equilibrator. The equilibration conditions in the CIC dual equilibrator were targeted at 37.0°C, pH 7.40 (balanced by the addition of NaHCO3 or HCl), and a Pco2 of 40.0 Torr. The blood samples were equilibrated over a range of gas flows for N2 and O2 in a stepwise manner to produce P02 ranging from 0 to 500 Torr. At each step, the blood samples were equilibrated for 3–5 min before 1 ml was withdrawn and analyzed on an IL 682 CO-oximeter programmed with porcine coefficients and a calibrated IL 1610 blood-gas analyzer. As the equilibrated samples were consumed from each chamber over 20–30 min, the blood in the equilibrating chamber was replenished with fresh heparinized blood from the anesthetized swine. After the final measurements at no O2 flow, complete deoxygenation was ensured by the addition of 20 mg sodium dithionite. Fresh human blood samples were used in similar methods to measure HbO2 saturation on an IL 682 programmed with human coefficients at PO2 levels ranging from 20 to 120 Torr. These measurements were used as a validation data set for the human mathematical modeling.

Statistical analysis. Data are presented as means ± SD. The linear regression analysis was used to determine the correlation between the IL 682 porcine hemoglobin saturation and the mathematical model-predicted porcine saturations. Agreement between saturation measurements made by the IL 682 CO-oximeter and the porcine HbO2 saturations predicted by the mathematical model were evaluated by use of Bland-Altman analysis (1, 17). In this analysis, the mean of the two measurements is plotted against the difference in the measurements. The limits of agreement of the two techniques is reported as the mean difference ± 2 standard deviations of the mean difference.

RESULTS

Mathematical modeling Hg dissociation curves using data sets. At pH 7.4, 37°C, the following basic porcine HbO2 dissociation equation was obtained from the porcine data points (n = 213).

\[
\%/(100) = (0.13534 \cdot P_{O2}^{3.02})/((0.13534 \cdot P_{O2}^{3.02} + 91.2) \cdot 100)
\]
The obtained fit was excellent ($r^2 = 0.98$, SE of the estimate = 7.1%), and there was no significant auto-correlation detected by use of the Durbin Watson statistic. This mathematical model with the correction factors provides a highly descriptive estimate of porcine hemoglobin saturation over the entire physiological range of $P_{O_2}$ values as well as those experimental extreme values that occur during hypoxemia, hemorrhage, and/or resuscitation. From the porcine model, the predicted $P_{50}$ was 32.9 Torr. This is consistent with the reported adult porcine $P_{50}$ of 32–34 Torr (4, 11, 13, 15, 24, 25). In addition, the predicted 99% ($P_{99}$) and 7.5% saturation with molecular oxygen ($P_{7.5}$) (the extremes) were, respectively, 150.8 and 14.4 Torr at 37°C and pH 7.4.

The descriptive modeling of the adult human $O_2$ dissociation curve on the 135 published measurements performed at 37°C and pH 7.4 yielded the following equation

\[
\frac{\%}{100} = \frac{(0.13534 \cdot P_{O_2})^{2.62}}{[(0.13534 \cdot P_{O_2})^{2.62} + 27.4]}
\]

Comparison of the human mathematical model with the validation set of human Hb saturations ($n = 95$ saturation determinations) measured on the IL 682 CO-oximeter programmed with human coefficients revealed a high linear correlation between both measurement methods with minimal variability ($r^2 = 0.98$). The $P_{50}$ predicted by the human model for the adult human was 26.1 Torr. This $P_{50}$ value was within the range of the values for humans (2, 12, 23). However, it is appreciably lower than the $P_{50}$ of the adult pig obtained from Eq. 2. At the extremes, the $P_{99}$ and $P_{7.5}$ for the adult human were, respectively, 146.9 and 9.7 Torr. This modeling predicts that, compared with the porcine $O_2$ dissociation curve, the human $O_2$ dissociation curve would rise faster at $P_{O_2} > 10$ Torr and thus reach a half-saturation value at a substantially lower $P_{O_2}$.

$P_{O_2}$ predicted and measured $HbO_2$ saturation data.

We performed 366 separate porcine $HbO_2$ saturation determinations on the IL 682 CO-oximeter. The temperature maintained in the dual-chamber equilibrator was 37.1 ± 0.1°C. The $P_{O_2}$ values measured by the IL 1610 ranged from of 639 to 0 Torr. The measured pH was 7.41 ± 0.03, and $PCO_2$ was 39.8 ± 0.08 Torr.

Figure 1 compares measured (IL 682 CO-oximeter) $HbO_2$ saturation with the corresponding calculated hemoglobin saturations from the measured $P_{O_2}$ (IL 1610) for both porcine and human mathematical models. At a $P_{O_2} > 100$ Torr, the human and porcine $HbO_2$ dissociation curves are essentially identical. However, as the $P_{O_2}$ declines below 80 Torr, the IL 682 CO-oximeter-measured and the mathematical model-predicted porcine $HbO_2$ saturations are noticeably shifted to the right of the predicted human $HbO_2$ saturations. Thus the porcine $HbO_2$ saturation decrease more rapidly, resulting in a $P_{99}$ at ~33 Torr compared with the predicted human $P_{50}$ of ~26 Torr.

Figure 2 shows porcine $HbO_2$ saturation measured with the IL 682 CO-oximeter with the $HbO_2$ saturation calculated from the measured $P_{O_2}$ using the porcine and human model. When measured porcine $HbO_2$ saturations are compared with saturations predicted by the porcine mathematical model, there is an extremely high degree of correlation between the data with minimal variability ($r^2 = 0.99$, SE of the estimate = 5.2%). Comparing measured $HbO_2$ saturations with saturations predicted by the mathematical model of the human $HbO_2$ saturation curve revealed a nonlinear pattern of correlation throughout the range of hemoglobin saturations in which measured porcine saturations were consistently lower than those predicted by the human mathematical model.

Figure 3 compares hemoglobin saturation differences with the use of porcine $HbO_2$ saturation data obtained from the IL 682 CO-oximeter and the differences in that data with saturations predicted from the
Fig. 3. Bland-Altman analysis of the IL 682 CO-oximeter porcine HbO2 saturation data and the HbO2 saturation data from the mathematical model specific for porcine HbO2 saturation. The IL 682 data is biased slightly lower (−0.4 vol%), and the difference in measurements between a saturation of 0–100% is <1.5 vol%. In contrast, if the HbO2 saturation of porcine blood is determined by human-specific methods, the difference could be as large as 18 vol%. Because the largest differences occur in the Po2 range of venous blood and the mathematical determination of O2 content is highly dependent on the HbO2 saturation, there exists a large potential for error in venous O2 content and thus global or regional O2 consumption when human-specific methods are used to determine the HbO2 saturation of porcine blood.

PO2 by the mathematical models in a Bland-Altman-style plot. Figure 3 shows that, over the range of saturation measurements, IL 682 measurements were biased slightly lower (mean Hg saturation bias = −0.4%), and the limits of agreement (±2 SD) were 0.7 and −1.5%. In general, this shows a good agreement of the two methods for determining the O2 saturation of porcine hemoglobin. In contrast, the Bland-Altman comparison of the IL 682-measured porcine HbO2 saturation levels with the HbO2 saturation predicted by the human mathematical model shows an average overestimation of the percent porcine HbO2 saturation by the human HbO2 saturation model of 9.8 ± 6.6. Between HbO2 saturations of 60 and 30, these differences represent a 20–40% error in the saturation determination. These differences in the HbO2 saturation are significant because they occur at the physiologically relevant mixed venous O2 tensions of 40–25 Torr.

**DISCUSSION**

The important new information presented in this paper is the development of a convenient, but solely descriptive, mathematical model for nonlinear estimation of porcine HbO2 saturation and the agreement with a porcine-specific CO-oximeter for laboratory determination of fractional HbO2 saturation. We developed this as a descriptive nonlinear cooperative rectangular hyperbola that describes the saturation curve accurately at both extremely low and high Po2 as well as in the physiological Po2 ranges. Because this is an entirely pragmatic and descriptive approach, the model does not have implications for the regulation of O2 binding by hemoglobin. However, for the first time, our model describes the entire saturation curve accurately, both at extremely low and high Po2 as well as in the middle. This accurate description and measurement of porcine HbO2 saturation is important because swine are frequently used for experimental situations (hemodilution, systemic hypoxia, ischemia-reperfusion, or resuscitation) that routinely produce extremely low or high blood oxygationation levels that result in extremely very low or near 100% hemoglobin saturations. To estimate with acceptable accuracy the delivery and consumption of O2 under such conditions, a complete HbO2 dissociation curve and measurement modality is required. The importance of accurate species-specific measurement techniques is demonstrated in the differences in the porcine-specific HbO2 saturation and saturation determined by use of human-specific methods.

One other purpose of this study was to validate the IL 682 CO-oximeter for accurate measurement of porcine HbO2 saturation. The validation of the IL 682 measurements is based on a Bland-Altman statistical method for evaluating differences in measurements obtained on the same samples by two different measurement techniques (1, 17). This method evaluates the mean difference between the two methods over the range of interest (estimated bias) and the variability of the measurements (standard deviation) around the mean difference. The mean difference ± 2 SD is defined as the “limits of agreement.” If no significant differences in data outcomes are produced between the two measurements within the range of interest, the methods can be used interchangeably. Because the existing methods of measuring porcine HbO2 saturation are extremely time consuming, we modeled the entire adult human and porcine O2-dissociation curves on the basis of data compiled from the literature by using published data from Bartels and Harms (4) and both published and unpublished data from Kim and Duham (15). This produced nonlinear cooperative mathematical models that were not based on the original Hill formula and are, therefore, purely descriptive. The models were nonlinear rectangular hyperbolas with positive cooperativity that described the HbO2 saturation for both adult humans and swine with high fidelity; they were also well defined at Po2 = 0 Torr and proved to be accurate at physiological extremes, i.e., both at low (<20 Torr) and high (>100 Torr) Po2 levels. We compared the predicted model data with directly measured values by using CO-oximetry. This validation produced essentially identical curves over the entire Po2 range of clinical and experimental interest. From Fig. 3 there is a close agreement of HbO2 saturation measured with the IL 682 CO-oximeter and the mathematical model of porcine HbO2 saturation. In addition, the limits of agreement (±1.1%, 2 SD) between the two methods would not be expected to cause any appreciable error in the calculation of O2 content or O2 consumption. Overall, the measured HbO2 saturation is slightly lower than the HbO2 saturation predicted by the mathematical model (−0.4% mean bias). However, this difference and the limited variability shown by the limits of agreement are experimentally and physiologically insignificant.
The main reason for the close agreement of these two different methods of porcine HbO2 saturation is related to the accuracy of the primary measurements. The measurements made by the IL 682 CO-oximeter are derived from the porcine-specific coefficient set stored in memory. This coefficient set is an inverse $4 \times 4$ matrix of the relative absorption coefficients at the measuring wavelengths of the IL 682 CO-oximeter. The IL 682 CO-oximeter uses this coefficient set and the absorption of the sample at the CO-oximeter wavelengths to calculate a fractional HbO2 saturation by dividing the HbO2 saturation by the sum (100%) of the oxy-, deoxy-, carboxy-, and methemoglobin saturation. The coefficient set is derived from porcine blood by using standard techniques by IL personnel (data on file with IL). In brief, the absorption coefficients for oxy-, deoxy-, carboxy-, and methemoglobin for porcine blood at the set wavelengths are determined by measuring a sample containing only one hemoglobin species. The absolute accuracy of the absorption coefficients for the individual hemoglobin is related to the assumptions made by IL in the protocols that generate the data for the calculation of the coefficients for the IL 682 CO-oximeter. Those protocols use fresh animal blood to generate “pure” hemoglobin species to determine the absorbance characteristics. However, pure deoxyhemoglobin is still potentially contaminated by the unknown small amount of carboxyhemoglobin in the fresh blood sample. The accuracy of the pure carboxyhemoglobin absorbance at the four wavelengths is limited by the amount of methemoglobin in the original sample. Finally, the accuracy of the absorbance characteristics of 100% HbO2 is limited by the small amounts of carboxy- and methemoglobin in the fresh sample. Before calculation of the coefficient set, the IL protocols correct the saturation measurements for the residual levels of carboxy- and methemoglobin. However, the polarographic and spectrophotometric experiential data used for modeling accounted only for oxy- and deoxyhemoglobin and do not measure or modify for contamination by carboxy- and methemoglobin. Although the small amount of these contaminants inherent in the fresh porcine blood has a small effect on the overall calculations, it does limit the absolute accuracy of the CO-oximeter and the original measurements by Kim and Duhm (15) and Bartels and Harms (4). Another potential small source of error in the measurement of the HbO2 saturation in the porcine blood is the use of HCl to correct the pH to 7.4. Because Cl competes for binding sites with 2,3-bisphosphoglycerate and CO2, it can decrease HbO2 affinity (20, 21). However, in this experiment the swine were anesthetized, ventilated, and maintained normothermic between arterial blood sampling. In addition, because the pH of the fresh arterial blood from the swine ranged between 7.39 and 7.42, pH correction was infrequent. Thus, although not fully documented, the impact of changes in the Cl content on the IL 682 measured HbO2 saturation was probably minimal.

The second purpose of this study was to estimate the error incurred, if human O2 dissociation curves are used in porcine experiments. There are substantial differences between the human and the porcine saturation data in the Po2 for venous blood samples. The magnitude of this difference is illustrated in the calculation of O2 content for regional or global O2 consumption. Because the HbO2 saturation is a major factor in the determination of O2 content $[(1.34\cdot Hb\cdot HbO2\ saturation) + (0.003\cdot \text{Po}2)]$, the differences in the HbO2 saturation and thus the O2 content determined by using the human methodology for porcine HbO2 saturation can be quite large. On the other hand, the differences in calculated arterial O2 contact are small (0.5%) because the porcine HbO2 saturation is overestimated by the human methodology difference <0.5 vol% at Po2 levels >100 Torr. However, if the Po2 of the venous blood is 40 or 30 Torr, i.e., in the physiological range, the HbO2 saturation derived from human methods is overestimated by 11.0 and 15.9 vol%, respectively. This leads to an overestimation of the venous O2 content in the porcine blood with normal Hb levels (10 g/dl) by 1.5 and 2.1 ml/dl, respectively. In these examples, the systematic error leads to an underestimation in the arterial-venous O2 content difference by 30.3 and 27.5%, respectively. This error is further compounded by the multiplication of the arterial and venous O2 content difference by the flow rate in the calculation of either regional or global O2 consumption. In addition, if the HbO2 saturation measurements are used to calculate the cardiac output by the Fick method, erroneously high HbO2 saturation measurements determined for porcine blood by using human-specific methods will result in a 45 and 43% overestimation in the cardiac output at venous Po2 levels of 40 or 30 Torr, respectively.

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