Investigating the hemoglobin of white rhinoceroses (Cerathotherium simum simum) and possible implications for anesthesia.

Ray L. Ball
Tampa’s Lowry Park Zoo, 1101 W. Sligh Ave., Tampa, FL USA 33604

Introduction:
Respiratory depression is potentially the most significant complication surrounding the anesthesia of both free-ranging and captive white rhinos. Large mammals in general experience cardiopulmonary depression and perfusion-ventilation (V/Q) mismatching. Specific complications are hypoxemia, hypercapnia, and acidosis. In spite of these potential complications, morbidity and mortality related to anesthesia are rare and numerous examples of prolonged anesthesia in this species are reported in the literature and anecdotally. White rhino hemoglobin has a known higher affinity for oxygen as is expected in large mammals. This hemoglobin also has some unique beta chains that is are unique to mammals and more closely related to fish. We suggest that some of the unique properties may influence how routine blood gas analysis is interpreted and that white rhinos under anesthesia are not as severely compromised as routine measurements may suggest. This has profound impact as effects to correct these perceived problems may lead to other complications.

A series of anesthetic events in two seven year old captive born white rhinoceros males was performed at Busch Gardens Tampa, Florida. Both were anesthetized for electro-ejaculation five times and had blood gas sampling at each event. Arterial blood was collected from an auricular artery on the inside of the ear using a heparinized 25-gauge butterfly set attached to a 3.0-ml heparinized plastic syringe. Blood gas and pH analyses on arterial blood samples were performed within 30 min of collection on a portable analyzer (AVL OPTI Critical Care Analyzer, AVL Scientific Corp., Roswell, Georgia 30077, USA). Typical blood gas analysis from these procedures is given in Table 1. Overall hypoxia, academia, and hypercapnia were common and often considered life threatening. On some occasions, blood gas values were below 7.0. Supplemental oxygen was provided at each procedure and heart rate and respiratory rates were within acceptable parameters (Radcliffe and Morkel 2007). Pulse oximetry readings for oxygen saturation of hemoglobin ranged from 45-80%. In spite of these findings, each procedure resulted in a complete recovery without complications. Findings were similar to those reported in wild caught rhinos (Bush 2004) but even more extreme.

In order to better understand the disparity in the clinical outcome with the blood gas analysis, a clinical investigation was made with awake, standing rhinoceroses. Blood gas values paired with pulse oximetry were collected from one adult male, two adult females, a 2 yr old juvenile and a 6 month old female. Venous blood samples were collected due to the ease of collection and measured as the samples above. Pulse oximeter reading including pulse and oxygen saturation of hemoglobin was taken. A manual pulse rate was obtained either by a stethoscope auscultation of the heart or a digital pulse from the tail. These findings are summarized in Table 2 along with a comparison from the literature (Citino 2007). Whole blood in heparin was collected to isolate white rhinoceros hemoglobin as described by Bauer and Pacyna using gas chromatography. A
adult female and the 6 month old calf from above were utilized in this assay. Carbon monoxide was used as a first step to generate absorbance spectra of white rhinoceros carboxyhemoglobin due to complications involved in using oxygen (Larson 2007, Wagman 2008). Absorbance spectra of HbCO in white rhinos was found to have a Sorbet band at 419nm. Normalized absorbance for titrated CO was higher in the adult female compared to the female calf.

Discussion and Conclusions:

The oxygen saturation derived by blood gas analysis was substantially different than that derived by pulse oximetry in the adult awake rhinoceros but there is good correlation between the calf for these values. The 2 year old juvenile lies in between these groups. It is possible that the pulse oximetry readings reflect the differing hemoglobin affinity for oxygen in the different size classes of these rhinoceros with the calf hemoglobin most closely fitting that of humans or domestic horses. The differences in oxygen saturation in this group of rhinoceros compared to Citino are difficult to explain but may be due to differences in sampling. PaO2 was shown to increase over time within 5 minutes of collection in iced water samples (Deane et al. 2004; Picandet et al. 2007) and to increase (Picandet et al. 2007) or decrease in samples kept at room temperature (Deane et al. 2004), so perhaps handling can explain these differences. The reported values of the anesthetized rhinoceros during electro-ejaculation are also not sensible in that that prolonged hypoxia and acidosis of this magnitude would be expected to result in longer term complication and potentially even death in the worst cases. Interestingly, SpO2 readings of 88-100% were obtained on several occasions on a white rhinoceros in dorsal recumbency for abdominal laparotomy, although readings of less than 94% predominated (Valverde 2010). All of these clinical findings and contradictions add support to the fact that differences in white rhinoceros hemoglobin have been documented (Baumann 1984). All of the values reported on the blood gas analyzer used are calculated from the algorithms of hemoglobin reflectance based on human oxygen dissociation curves (ODC) and hence the need for more specific ODC for large mammals and especially one with such a unique hemoglobin. White rhinoceros hemoglobin does not appear to be responsive to organic anions (2, 3 DPG or ATP) nor to CO2 (Baumann 1984). It is speculated that the ODC for white rhinoceros may closely resemble that of humans without any 2,3 DPG added. The development of a carboxyhemoglobin spectrum is the first technical step towards developing this ODC for white rhinoceros and future efforts will try to finalize this. Future work will now focus on determining an equilibrium binding constant.

References:


Table 1. Examples of blood gas values from white rhinoceros anesthetized for electroejaculation.

<table>
<thead>
<tr>
<th>White rhinoceros #1</th>
<th>pH</th>
<th>pCO2 mmHg</th>
<th>pO2 mmHg</th>
<th>sO2 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>7.333</td>
<td>64.6</td>
<td>47.4</td>
<td>82.3</td>
</tr>
<tr>
<td>T15</td>
<td>7.32</td>
<td>59.7</td>
<td>41.8</td>
<td>76.6</td>
</tr>
<tr>
<td>T30</td>
<td>7.345</td>
<td>64</td>
<td>41.1</td>
<td>79.9</td>
</tr>
<tr>
<td>T45</td>
<td>7.404</td>
<td>49.9</td>
<td>43</td>
<td>78.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>White rhinoceros #2</th>
<th>pH</th>
<th>pCO2 mmHg</th>
<th>pO2 mmHg</th>
<th>sO2 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>7.21</td>
<td>92.9</td>
<td>28.9</td>
<td>43.9</td>
</tr>
<tr>
<td>T15</td>
<td>7.089</td>
<td>83.3</td>
<td>45.4</td>
<td>75</td>
</tr>
<tr>
<td>T30</td>
<td>7.154</td>
<td>85.3</td>
<td>35.3</td>
<td>59.2</td>
</tr>
<tr>
<td>T45</td>
<td>7.235</td>
<td>79.2</td>
<td>33.4</td>
<td>58.5</td>
</tr>
</tbody>
</table>
Table 2. Pulse oximetry readings and venous blood gas analysis of standing, awake white rhinoceros and comparison to published values.

<table>
<thead>
<tr>
<th></th>
<th>Adult rhinoceros</th>
<th>2yr old juvenile</th>
<th>6 month old calf</th>
<th>Citino 2007 (Arterial)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>Pulse Ox %O2</td>
<td>68.8</td>
<td>3.839</td>
<td>15</td>
<td>75.6</td>
</tr>
<tr>
<td>Pulse Ox Pulse (bpm)</td>
<td>60.133</td>
<td>11.25</td>
<td>15</td>
<td>79.2</td>
</tr>
<tr>
<td>Digital pulse (bpm)</td>
<td>59.53</td>
<td>10.09</td>
<td>15</td>
<td>78</td>
</tr>
<tr>
<td>Blood gas (V)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.361</td>
<td>0.024</td>
<td>15</td>
<td>7.346</td>
</tr>
<tr>
<td>pCO2 mmHg</td>
<td>53.833</td>
<td>1.084</td>
<td>15</td>
<td>51.98</td>
</tr>
<tr>
<td>pO2 mmHg</td>
<td>76.38</td>
<td>6.68</td>
<td>15</td>
<td>51.52</td>
</tr>
<tr>
<td>sO2 %</td>
<td>93.74</td>
<td>1.182</td>
<td>15</td>
<td>86.74</td>
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White rhinoceros anesthesia

- Ultra-potent narcotics
  - Etorphine
  - Carfentanil
- Sedatives
  - Alpha 2
  - Azaperone
- Detomidine/Butorphanol
- Medetomidine/Butorphanol
White rhinoceros anesthesia complications

- Hypoxia
- Acidosis
- Hypercapnea
- Tachycardia
- Hypertension

- Mortality uncommon in NA
- Prolonged procedures

Anesthetic management of a white rhinoceros (*Ceratotherium simum*) undergoing an emergency exploratory celiotomy for colic

Alexander Valverde*, Graham J Crawshaw†, Nicola Cribb*, Maria Bellei*, Giacomo Gianotti*, Luis Arroyo*, Judith Koenig*, Maya Kummrow*,† & Maria Carolina Costa*

*Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada
†Toronto Zoo, Scarborough, ON, Canada
Series of immobilizations for ejaculation

- Etorphine induction
  - ~4mg
- Alpha 2 (xylazine)
- Supplemental oxygen
- Electro-ejaculation
- NSAIDs
- Blood gas via auricular artery
Immobilization for ejaculation

- HR 50-72 at rest
  - 100 when stimulated
- RR 6-24
- SpO2 45-80%
- Various drugs used to try and improve blood gas findings (n=4)
  - Dopram
  - Naloxone
  - NaHCO3

- All recovered well
- Rhinos given stimulants all constipated for 24-36 hrs post-recovery
## Typical blood gas analysis

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How did these rhinos with severe hypoxia and acidosis survive??

• Induction?
• Methods of ABG collection?
• Effect of electro-ejaculation?
• White rhinoceros hemoglobin and instrumentation?

White rhinoceros hemoglobin
Baumann 1984

- Glutamic acid at Beta 2 position
- Little effect from CO2, ATP and 2,3 DPG
- Similar to fish Hb
- High affinity for oxygen
  - $P50 = 17\text{mmHg (2.67kPa)}$
- Trend for larger mammal to have high O2 affinity

- Can a clinically useful algorithm be developed for SpO2 and ABG?
- Can a ODC for white rhinos be developed?
- Does myoglobin have a role??
Measure awake rhinoceros

- 1.2 adults (n=15)
- 0.1 2 year old (n=5)
- 0.1 6 month old (n=5)
- Venous BG samples
- Heart rates
- Pulse oximetry readings
## VBG and SpO2 from standing awake rhinoceroses

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</table>
VBG and SpO2 from standing awake rhinoceros

• SpO2
  – Differences larger in adults
  – Calf similar to horse/human

• SaO2 close to the same
  – pH calculated from this

• pO2/pCO2 venous vs. arterial

• pCO2 higher than horses

• pH 7.35-7.45
  • pCO2 35-45 mmHg
  • pO2 >79 mmHg
  • CO2 23-30 mmHg
  • SO2 >94%

• pH 7.31-7.41
  • pCO2 41-51 mmHg
  • pO2 30-40 mmHg
  • CO2 23-30 mmHg
  • SO2 75%
Valverde 2010

• SpO2 between 88% and 100% saturation
• Values for blood gases were probably not accurate for PaO2 due to handling temp
  – Still reflect progressive hypoxia, acidosis, hypercapnea
• SpO2 values overestimated SaO2
  – Other reports SpO2 underestimates SaO2
• Conflicts about SpO2 and ABG reliability
CO binding properties of white rhinoceros.

- Wagman, J., R. Larson, C. Reimink, R. Ball.
  - Preliminary work shows a higher absorbance for CO in adult rhinos
    - Suggest higher affinity for O2
  - Adult hemoglobin differs from juvenile rhinoceroses
Significance

• PaO2 <60mmHg hypoxemia in horses
  – SaO2 93%
• Apply to white rhinoceros??
• Redefine hypoxia in white rhinoceros
  – Prevent unnecessary treatment
  – Opioids and constipation
17mmHg Baumann 1984
97% at 98mmHg Citino 2007
90% SaO2 = 30 mmHg

Myoglobin?
Summary

• White rhino ODC will be shifted to the left
  – P50 17-20mgHg
• Organic anions have little affect on oxygen binding
• Define hypoxia for WR at SaO2 ~30mmHg
• Juvenile rhinos may have ODC similar to humans and horses
Anesthetic management

• Always provide oxygen
• Do not worry about CO2
• Interpret ABG values with caution
• Usefulness of ABG ??

Fig. 1: Drawing of a recumbent rhinoceros showing the placement of the nasotracheal tube passing through the larynx into the trachea, with the tube attached to an oxygen tank with a pressure regulator and a flow meter to control the O₂ flow.

Acknowledgments

• Rhino Staff @ Busch Gardens
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• Lowry Park Zoo