Red Blood Cell Metabolism and Hemoglobin Oxygen Affinity.  
Effect of Vinbunrine on Normobaric Hypoxic Rats

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Received December 18, 1998; accepted May 21, 1999

The influence of Vinbunrine on red blood cell metabolism and hemoglobin oxygen affinity was investigated in normobaric rats (FIO2: 10%). Two periods of hypoxic exposure were performed (10—21 d). In each experimental series, rats were divided into two groups: Treated rats received a daily intraperitoneal injection of drug (4 mg/kg). Control rats in the same conditions of hypoxia received an isotonic saline solution.

For a 10 d exposure period, Vinbunrine does not affect red blood cell metabolism nor hemoglobin oxygen affinity. At 21 d period exposure, 2, 3 diphosphoglycerate (2, 3 DPG) and ATP amounts increase in treated rats. The rate of increase was 10% (p<0.05) and 28% (p<0.01), respectively. Red blood cell metabolism effect of Vinbunrine was not accompanied by a modification in affinity hemoglobin oxygen (Hb-O2); no statistically significant difference was observed between treated rats and control rats concerning p50 (partial pressure oxygen at half hemoglobin saturation).

Results suggest that Vinbunrine has a metabolic effect corresponding to a glycolysis anaerobic stimulation, which can improve oxygen delivery to tissues and could explain the favorable hemoreologial action of Vinbunrine observed in a previous investigation.

Key words normobaric hypoxia; Vinbunrine; red blood cell metabolism; affinity hemoglobin oxygen

The treatment of cerebrovascular ischemic diseases and disorders associated with aging remains a major clinical problem. A common factor of these diseases is a decreased oxygen delivery causing a cerebral hypoxia. An alternative approach of cerebrovascular pathology involves treatment with oxygen and improves its delivery to brain. The oxygen transport from lung to tissues is essentially due to red blood cell (RBC) hemoglobin. The oxygen hemoglobin binding is represented by the oxygen dissociation curve (ODC). The shape and position of ODC is described by the oxygen pressure at half hemoglobin saturation (p50) and depends upon several factors: pH, pCO2, temperature and 2, 3 diphosphoglycerate (2, 3 DPG) concentration.

2, 3 DPG is a RBC metabolite, which lowers the oxygen affinity of hemoglobin and facilitates oxygen unloading at the tissue level.

Vinbunrine is an alkaloid derived from Vinca minor and has been shown to prevent the lethal consequences of hypoxic hypoxia. It possesses a cerebral oxygenator activity increasing brain glucose consumption and mitochondrial cytochrome activity without lactate production. Furthermore Vinbunrine improves learning and memory processes disrupted by several pharmacological manipulations and hypoxia in mice and rats. The effects of drug would be linked to biochemical and morphological protection resulting from an improvement in cerebral oxygenation.

Among mechanisms favouring cerebral oxygenation, an action on blood red cell metabolism and/or haemoglobin oxygen affinity could be implicated. In our investigation we have studied the eventual effect of Vinbunrine on ATP and 2, 3 DPG concentration in rats placed in normobaric hypoxia approaching physiopathological current conditions.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 350—400 g (15 or 16 weeks old) were purchased from Ifa-Credo (France) and housed in animal cages for a minimum of 7 d prior to the experimental study with food and water available ad lib.

Experimental Design The normobaric hypoxia was conducted in glass doored chambers. The O2 concentration (FIO2 = fractional inspired O2) in chambers was maintained at 10% by mixing air and azote. Gas composition was continuously monitored with a rapid O2 analyzer (Capnograph, France). Carbon dioxide concentration was less than 0.5% during the experiment. The temperature was maintained at 20—22°C and pCO2 at 40 mmHg. Two periods of exposure were assessed: 10 and 21 d. For each period, two groups of rats were placed in the chambers. Treated rats received an intraperitoneal injection of 4 mg/kg/d of Vinbunrine. Control rats received normal saline solution.

Biochemical Determinations After appropriate periods of exposure, the rats were killed by decapitation and whole blood was collected in ice-cold heparinized tubes. Haematocrit (Hct) of samples was determined by microhematocrit centrifugation (10000 rpm).

ODC were determined using a Hemox Analyzer TCS (U.S.A.). This is a continuous method combining a dual wavelength spectrophotometer and a Clark oxygen electrode. The blood samples were suspended in a buffer solution (Tris, pH 7.40) and blood oxygen affinity was analyzed as p50: partial pressure of oxygen in mmHg required for 50% saturation of hemoglobin. For RBC metabolite determination, blood was deproteinized by addition of trichloroacetic acid. The supernatant obtained was used. 2, 3 DPG concentration was measured spectrophotometrically. Reagents were supplied by Sigma. Results were expressed as µmol/ml RBC. ATP determination was performed by spectrophotometric method using reagents supplied by Sigma (366 UV Kit), and results were expressed as µmol/l RBC.

Statistical Analysis The data shown are the mean values±standard deviation (S.D.). Mann and Whitney U-

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tests (non parametric test) was used for the comparison between groups of rats in each experimental series. A value $p<0.05$ was considered to indicate significant difference.

RESULTS AND DISCUSSION

10 d Hypoxic Exposure Results corresponding to this experimental series are represented in Table 1. Comparison between treated rats and control rats does not show any statistically significant difference concerning RBCs metabolite (ATP-2, 3 DPG) nor p50. A small increase in ATP levels of treated rats was observed (not significant).

<table>
<thead>
<tr>
<th>Normoxic rats (n=10)</th>
<th>Hypoxic exposure for 10 d</th>
<th>Hypoxic exposure for 21 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct (%)</td>
<td>Control (n=10)</td>
<td>Treated (n=10)</td>
</tr>
<tr>
<td>44.1 ± 1.9</td>
<td>63.7 ± 0.7</td>
<td>62.9 ± 0.9</td>
</tr>
<tr>
<td>p50 (mmHg)</td>
<td>38 ± 1</td>
<td>39.3 ± 0.8</td>
</tr>
<tr>
<td>ATP (µmol/RBC)</td>
<td>841 ± 57</td>
<td>915 ± 52.6</td>
</tr>
<tr>
<td>2, 3 DPG (µmol/ml RBC)</td>
<td>5.11 ± 0.4</td>
<td>7.29 ± 0.26</td>
</tr>
</tbody>
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*p < 0.05, ** p < 0.01.

21 d Hypoxic Exposure Results are shown in Table 1. In this experiment series RBCs metabolite levels increased in treated rats. Vinbunarine produced 28% more ATP than control rats ($p<0.01$) while 2, 3 DPG amounts in treated rats was 10% higher than control rats ($p<0.05$).

The p50 and Hct values of treated rats were not significantly different from those of control rats.

Hypoxia effect on RBC metabolism and affinity hemoglobin oxygen (Hb-O$_2$) was previously studied, and under hypoxic conditions there is essentially enhancement in 2, 3 DPG concentrations.$^{2,10}$ This was confirmed in our investigation where hypoxia control rats presented elevated 2, 3 DPG amounts compared to normoxic rats. Concerning the pharmacological effect, results shown that Vinbunamine administration at a dose of 4 mg/kg/d during 10 d does not affect RBC metabolism nor affinity Hb-O$_2$ of rats placed under moderate normobaric hypoxic conditions (FiO$_2$ = 10%), while under the same conditions daily Vinbunamine administration for 21 d produced an increasing of RBC metabolism 2, 3 DPG and ATP concentrations (10% and 28%, respectively). Vinbunine effect on 2, 3 DPG concentration was observed in a previous investigation,$^{11}$ using a hypoxia/anoxia performed by an intra-peritoneal injection of sodium cyanate (KCN) which blockades the mitochondrial oxidative system and eliminates all O$_2$ utilization. We have selected an experimental design technique leading to moderate normobaric hypoxia which approaches an actual physiopathologic situation. Concerning the effect on RBC ATP amounts, an in vitro study has shown that Vinbunamine addition to conserved and experimentally aged RBC maintained a high enough ATP levels to preserve the discoidal RBC form.$^{11}$

To examine the impact of drug on affinity Hb-O$_2$ we measured p50. The results does not indicate any significant statistical difference between the two rat groups (treated versus control). Paradoxically, increasing 2, 3 DPG concentration did not affect affinity Hb-O$_2$. There could be two reasons for the lack of effect on p50 in our investigation: the increase of DPG is not sufficient to induce an ODC shift, or others effectors and elements, such as pH, CO$_2$ and Cl$^-$ could affect the p50 value.$^{12,13}$ This would be interesting to determine in a next study.

The concomitant augmentation of ATP and 2, 3 DPG amounts produced by Vinbunamine administration (4 mg/kg/d–21 d) in hypoxic rats would correspond to RBC glycolysis stimulation or glucose consumption by RBC probably activating one of three major enzymes (Pyruvate Kinase, Hexokinase, Phosphofructokinase).

These drug effects could explain the different favorable actions of Vinbunamine previously described: hemoreological improvement,$^{5}$ enhancement in cerebral O$_2$ consumption$^{6}$ and increase in cerebral metabolic activity.$^{14}$ Since 2, 3 DPG and ATP are highly energetic compounds and can improve tissue oxygenation either by their effect on affinity Hb-O$_2$ or by participating in RBC hemodynamic morphologic equilibrium.

REFERENCES