Fast Hemoglobins and Red Blood Cell Metabolites in Citrate Phosphate Dextrose Adenine Stored Blood*

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ABSTRACT

Blood was drawn from 10 fasted, healthy volunteers and stored under standard blood bank conditions in citrate-phosphate-dextrose-adenine (CPDA-1). Blood was sampled before storage (Day 0) and on Days 5, 12, 19, 26, and 35. Laboratory testing for glucose, HbA1c, pyruvic acid, lactic acid, adenosine triphosphate (ATP), 2, 3-diphosphoglycerate (2,3DPG), plasma free hemoglobin (Hb) and pH (blood gases) were performed. In addition, P50 was also serially measured in two of the individuals and in their stored blood. Significant elevations of HbA1c and HbA1c (fast hemoglobins) were found on Days 12 and 19 of storage (p < 0.05). These elevations of fast hemoglobins are due to hypoxia, acidosis, and hyperglycemia. Following the initial elevation of the fast hemoglobins (Hbs), there was a decline in their concentration, from Day 12, which could partly be explained by cell death.

Introduction

An interesting field was opened with the studies related to red blood cell (RBC) metabolism in stored blood. Different aspects of this metabolism have been studied including that of purine metabolism. Based upon results of these findings, researchers have attempted to prolong red blood cell (RBC) survival in stored blood by the addition of different precursors or their metabolites. Dihydroxyacetone has been introduced as an additive to the storing solution in order to maintain elevated levels of 2,3-diphosphoglycerate (2,3DPG), a vital component in hemoglobin’s (Hb) oxygen carrying capacity.

Along with the benefits that were derived from the addition of these substances, some potentially negative effects may result, such as the elevation of fast
Previous studies have shown that uremic and diabetic patients have elevated levels of HbAla+b and HbAlc. These elevations have been attributed to the metabolic environment of the RBC. This environment is characterized by acidosis, hypoxia and hyperglycemia. Blood stored over 35 days in vitro presents a similar “milieu” to that of uremic and diabetic patients. The purpose of the study was to elucidate the quality of stored blood with regard to its fast Hbs.

Materials and Methods

Blood was collected from 10 fasted volunteers into plastic bags containing CPDA-1 and stored for 35 days under standard conditions. The composition of CPDA-1 is sodium citrate, 1.66 g; monobasic sodium phosphate, 140 mg; citric acid, 206 mg; dextrose, 2 g; and adenine, 17.3 mg in a volume of 63 ml. The scheduling for sampling was as follows: before storage (day 0), and after storage on days 5, 12, 19, 26, and 35. All of the volunteers were considered healthy, as shown by their normal values on day 0 (table I). Aerobic and anaerobic cultures were taken from the stored blood on Day 35 and showed no growth. This indicated that the metabolic changes detected were not the product of bacterial contamination.

The measurement of fast Hbs was done by high performance liquid chromatography (HPLC). Lactic acid and pyruvic acid, ATP, and plasma free Hb, were performed by standard spectrophotometric techniques. Blood gases were measured by electro­metric microgasometer and the P50 by means of a Co-oximeter. Glucose was measured by standard glucose oxidase technique. In addition, HPLC was the method selected for fast Hbs determinations because of its advantages: small amount of blood needed (6 to 10 microliters), coefficient of variation (5 to 10 percent), rapid elution, and the ability of automating the method. The Davis method as modified by Widness et al. was utilized. This method has the advantage of using overnight dialysis which allows measurement of the stable form of HbAlc. The HPLC station consisted of a Perkin-Elmer Series 2/2 pump, LC 75 variable wavelength spectrophotometer, and ISS-100 autosampler with refrigeration tray which was cooled to 4°C. Hemoglobin solutions were stored at −70°C when not analyzed immediately.

### Statistical Analysis

Analysis of variance, repeated measured design, and the paired T-test were used in our study. All our data and figures are mean values ± 1 SD.

#### TABLE I

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 12</th>
<th>Day 19</th>
<th>Day 26</th>
<th>Day 35</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbAla+b</td>
<td>2.4 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>3.0 ± 0.03</td>
<td>2.0 ± 0.2</td>
<td>2.5 ± 0.8</td>
<td>% Hb</td>
</tr>
<tr>
<td>HbAlc</td>
<td>6.0 ± 0.4</td>
<td>6.7 ± 0.7</td>
<td>7.9 ± 0.7</td>
<td>7.6 ± 1.0</td>
<td>6.9 ± 0.4</td>
<td>6.6 ± 1.0</td>
<td>% Hb</td>
</tr>
<tr>
<td>Glucose</td>
<td>76 ± 10</td>
<td>331 ± 188</td>
<td>329 ± 54</td>
<td>275 ± 42</td>
<td>213 ± 89</td>
<td>225 ± 19</td>
<td>mg/100 ml</td>
</tr>
<tr>
<td>Lactic A</td>
<td>8.2 ± 3.5</td>
<td>48 ± 12</td>
<td>89 ± 15</td>
<td>135 ± 10</td>
<td>230 ± 33</td>
<td>228 ± 53</td>
<td>mg/100 ml</td>
</tr>
<tr>
<td>Pyruvic A</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.5</td>
<td>3.1 ± 1.1</td>
<td>3.8 ± 0.6</td>
<td>1.9 ± 0.5</td>
<td>2.5 ± 0.6</td>
<td>mg/100 ml</td>
</tr>
<tr>
<td>Plasma</td>
<td>Free Hb</td>
<td>9 ± 5</td>
<td>13 ± 7</td>
<td>24 ± 12</td>
<td>20 ± 10</td>
<td>26 ± 11</td>
<td>40 ± 24</td>
</tr>
<tr>
<td>LA/PA</td>
<td>10 ± 2</td>
<td>72 ± 28</td>
<td>28 ± 36</td>
<td>36 ± 126</td>
<td>126 ± 94</td>
<td>10 ± 1.5</td>
<td>umol/gHb</td>
</tr>
<tr>
<td>ATP</td>
<td>4.2 ± 1.5</td>
<td>3.8 ± 1.0</td>
<td>3.5 ± 1.0</td>
<td>2.9 ± 1.0</td>
<td>3.0 ± 1.0</td>
<td>3.2 ± 1.0</td>
<td>umol/gHb</td>
</tr>
<tr>
<td>2,3 DPG</td>
<td>0.18 ± 0.08</td>
<td>0.15 ± 0.04</td>
<td>0.09 ± 0.03</td>
<td>0.04 ± 0.02</td>
<td>0.01 ± 0.004</td>
<td>0.007 ± 0.001</td>
<td>umol/gHb</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.03</td>
<td>6.91 ± 0.03</td>
<td>6.78 ± 0.02</td>
<td>6.68 ± 0.01</td>
<td>6.60 ± 0.02</td>
<td>6.55 ± 0.03</td>
<td>-log [H+]</td>
</tr>
<tr>
<td>PO2</td>
<td>31.0 ± 7.1</td>
<td>39.6 ± 6.6</td>
<td>39.3 ± 7.4</td>
<td>37.9 ± 7.0</td>
<td>40.3 ± 7.2</td>
<td>42.6 ± 7.2</td>
<td>mm Hg</td>
</tr>
</tbody>
</table>
Results

Elevation in HbAlc in stored blood (figure 1) has been shown by previous investigators.\(^\text{16}\) Our findings corroborate these results. However, this is the first study in which HbAlc has been serially sampled over time under standard conditions of CPDA-1 blood storage.

Elevation of HbAlc in our study occurred on days 12 and 19 (p < 0.05). In addition, HbAla+b was also found elevated on days 12 and 19 (p < 0.05, figure 2). Elevation of HbAla+b has not been previously described. Concomitant increases in the levels of pyruvic acid and HbAla+b were detected. During storage glucose concentration significantly declines as the levels of pyruvic and lactic acids increase (table I).

The level of ATP remained fairly stable. The levels of 2,3-DPG diminished very rapidly to insignificant values. Plasma free Hb increased and appeared to be a gross indicator of extensive cell death. The pH decreased as a result of lactic acid, pyruvic acid, and CO\(_2\) accumulation.

Measurement of the P50, in two of the individuals tested, showed significant decrement in linear relation to the decrease of 2,3-DPG. The PO\(_2\) values remained below 50 mm Hg during the study (table I).

Discussion

The measurement of fast Hbs, particularly HbAlc, serves as an aid for the management of patients with diabetes mellitus.\(^\text{2,18}\) Glycosylation occurs by a well described nonenzymatic reaction \textit{in vivo} and \textit{in vitro}.\(^\text{4,15}\) Properties of blood stored with CPDA-1 anticoagulant solutions are well described in a previous study.\(^\text{20}\) Glucose is actively consumed during storage.\(^\text{12}\) A recent study,\(^\text{16}\) as well as our own laboratory observations, show that HbAlc formation occurs in stored blood, and that this formation is related to the high concentration of glucose present in the final storage solution.

It has been shown that HbAla+b was elevated in Type 1 glycogen storage disease,\(^\text{19}\) uremia, and diabetes.\(^\text{8}\) The reason for this elevation is thought to be secondary to chronic hypoxia, metabolic acidosis, and a relative inhibition of pentose-phosphate shunt.\(^\text{8}\) The composition of HbAla+b has been partially deter-
mined, and one of its constituents, HbAla1, has been shown to be glucose-6-phosphate.5

The present study shows a significant elevation of HbAla+b and HbAlc in CPDA-1 stored blood in which lactic acidosis, hypoxemia, and hyperglycemia are here clearly found. Therefore, the environment for the RBC in the previously mentioned metabolic diseases and in CPDA-1 stored blood are similar, both promoting fast Hbs formation.

Previous reports of elevated HbAla+b in stored blood have not been described. In this report, HbAlc was analyzed by means of HPLC and, therefore, differs from the only previous report in the literature concerning non-enzymatic glycosylation of blood proteins in stored blood. In our study, the stored blood was studied at weekly intervals, during the entire storage period (35 days CPDA-1 in the blood bank). Finally, other biochemical parameters during storage were studied, which may help explain the elevation of fast hemoglobins in stored blood.

**Conclusion**

Both HbAla+b and HbAlc were significantly elevated in CPDA-1 stored blood, owing to hypoxia, acidosis and hyperglycemia (p < 0.05). It is speculated by the present authors that the decline of HbAla+b and HbAlc, after day 12, is due to preferential lysis in older cells (highest HbAla+b and Alc) and, hence, their loss in plasma removal when making the Hb solution. This red cell lysis is documented by increasing concentrations of plasma free Hb. However, full documentation of this speculation would demand red cell gradient (young cells/old cells) and subsequent chromatography.

The clinical significance of transfusion of stored blood is unclear at this time and requires further study. It is also speculated by us that the low 2,3-DPG is the most important factor affecting Hb-O2 transport, although HbAlc has been shown in vitro to be less reactive to 2,3-DPG than HbAo (adult hemoglobin).

Finally, it is our desire to make the clinical scientist aware of the changes in fast Hbs and metabolic content that occur in CPDA-1 stored blood over time.

**Acknowledgments**

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References