The study of carbon monoxide placenta blood hemoglobin oxygenation

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Abstract. With the great progress of hemoglobin-based blood substitutes, however, they all have a same problem that is hard to get effective stable structure of hemoglobin. The hemoglobin can be oxidized into met hemoglobin in the process of purification, lead to the loss of the function of carrying and transporting oxygen. The combination of CO and placenta blood hemoglobin ability is far higher than that of O2, the structure and properties of carbon monoxide hemoglobin (HbCO) has more stable. Red blood cell (RBC) is mixed with organic solvent for hemolysis and centrifuged for removal of stroma. To prevent MetHb formation during the procedure, Hb was carbonylated in advance. Excessive carbon monoxide pumped to hemoglobin, hemoglobin is converted into HbCO. HbCO is decarbonylated to regenerate HbO2 by exposing the solution to oxygen and visible light, the content of HbCO and HbCO is measured by blood gas analyzer. By comparing the liquid membrane and film boiling method, determination of oxygenation results. Measured liquid membrane oxygenation is more effective than hollow fiber module method. Through the liquid membrane method, large quantities of hemoglobin can be treated in production, it also can reduce the loss of hemoglobin during purification, increase oxygen affinity.

1. Introduction

The development of hemoglobin-based blood substitutes has now advanced to the stage that several products are being tested in clinical trial [1-2]. These studies, as well as numerous animal studies, have demonstrated that the administration of relatively large quantities of extracellular hemoglobin or hemoglobin derivatives may lead to a variety of undesirable toxic side effects [3]. It has been known for more than a decade that the administration of native hemoglobin causes severe nephrotoxicity’s, which are apparently associated with the excretion of hemoglobin dimers via the kidneys. Initially, it was suggested that at least some of these toxic effects may be due to impurities that remained present in the various hemoglobin preparations, such as endotoxins and/or stromal phospholipids [4-6]. It is proposed that this limitation can be overcome by increasing molecular size and oxygen affinity. We have established a new method by taking the advantage of excellent stability of carbonyl hemoglobin (HbCO), then remove CO from Hb before purification.

2. Materials and methods

Preparation of stroma-free HbCO

Washing hemoglobin: RBCs were obtained from Tianjin union stem cell gene engineering company. The placenta blood was put into centrifugal tube, Volume should not exceed 1/3 of centrifuge tube, added the physiological saline to two-thirds of the centrifugal tube, centrifuged by high-speed centrifuge table (≥2,800g, ≤15min, 4°C). The supernatant was removed after the end of centrifugation, repeat the process 3 to 5 times until the supernatant was close to colorless.

Carbonylation: The washed blood was transferred to the conical flask, added an equal volume of saline to dilute. The CO was pumped into the bloodstream by syringe needle, the time was about 30 min, and the whole process would find that the color of the blood changed from red to dull-red. The solution was centrifuged by high-speed centrifuge table (≥2,800g, ≤15min, 4°C), the supernatant was removed after the end of centrifugation.
Membrane remove: first, the centrifugal solution (RBC) was put into the ice water to cool to 4°C, then, RBC was mixed with CH2Cl2 for hemolysis, the volume of RBC and CH2Cl2 is 1:0.2. The mixed solution was shaken for 3 min and then centrifuged (28,000g, 15 min). An HbCO solution was separated as a top layer. After repeating the procedure again, residual CH2Cl2, in the HbCO solution was removed out at ca. 20 torr at 40°C in dark.

The oxygen of HbCO
Method 1: hollow fiber module method
COHb solution was frown through hollow fiber module, with the condition of light, ice water bath, oxygen, CO was removed from HbCO solution, it is purposed to stabilize hemoglobin.

Scheme 1: Blood goes around hollow fiber, the oxygen goes into the hollow fiber.
20 ml NaHCO3 was added into the 40 ml removed membrane solution to accommodate pH. The pH of HbCO was 8.25. According to Bohr Effect: with the loss of the pH value, the oxygen affinity of hemoglobin was receded. So control pH of HbCO fluid for weak alkaline. The test was started in a bottle, the content of HbCO and HbCO is measured by blood gas analyzer.

Scheme 2: Change the way of oxygen and blood circulation mode, other conditions are not changed. The oxygen goes around hollow fiber, blood goes into the hollow fiber.

Method 2: liquid membrane method
To make HbCO solution forming a very large area of liquid membrane on the surface, the gas exchange area of HbCO was increased, it is more favorable for CO and O2 exchange.

Scheme 1: pumping in oxygen and evacuated replacement
NaHCO3 was added into the 80 ml removed membrane solution to accommodate pH. The pH of HbCO was 7.8. The HbCO solution was transferred into an eggplant shaped bottle after pH adjustment, connected to the rotary evaporation apparatus for oxygenation in the condition of light and ice water bath. the whole process of experiment was need to access to oxygen, need a vacuum after 10 min oxygen flux, continuously repeating the experiment operation, every once in a while after taking 3 ml HbCO solution, diluted with 20 ml distilled water, with 0.22μm membrane filter. The content of HbCO and HbO2 were measured by blood gas analyzer.

Scheme 2: In combination with oxygen and evacuated replacement
Phytic acid 0.76 g and 0.76 g ascorbic acid were dissolved in 40 ml distilled water, adjusted the pH to 7.5, mixing with 40 ml removed membrane solution. In the 80 ml mixed solution, the final concentration of phytic acid was 10 mmol/L, the final concentration of ascorbic acid was 5 mmol/L. The mixed solution was transferred into an eggplant shaped bottle, connected to the rotary evaporation apparatus, and only used its rotation function for oxygenation with the condition of light and ice water bath. At the beginning of the experiment, the mixed solution was, then pumped in oxygen 10 min, vacuumized 10 min. Continuously repeating the experiment operation, every once in a while after taking 3 ml HbCO solution, diluted with 20 ml distilled water, with 0.22μm membrane filter. The content of HbCO and HbO2 were measured by blood gas analyzer.

3. Results and analysis

3.1 The result of hollow fiber module method
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The result of scheme 1:
At the beginning of reaction, the percentage of HbCO fell sharply, as the experiment progresses, the speed decreases, three hours later, the percentage was no longer dropped, eventually remained at around 82%, and the percentage of HbO₂ increased slowly.

The result of scheme 2:
Though the way of oxygen and blood circulation mode had been changed, the HbCO solution can contact with more sufficient oxygen, but on the whole the results did not have a material impact, the percentage of HbCO still no longer dropped after 81%.

By comparing the results of two schemes, the process of experiment was taken by two different ways, but got the same results. Two experimental schemes finally measured the percentage of HbCO was around 81%, cannot be further reduced. Method of hollow fiber module does not apply to HbCO oxygenation.

3. 2. The result of liquid membrane method
The result of scheme 1:
As we could see from figure 4, the percentage of HbCO dropped to 1.5%, HbO2 in quantity from 3.1% to 96.4%, and the percentage of increased quickly, HbCO was converted to HbO2 within 5 h. The metHb was 3.1%.

By comparing the results of two schemes, we found that the phytic acid was of a similar effect with 2, 3 - DPG, it can combine and promote the release of oxygen. Vc was strong reducibility, Hb could not be oxidized to high iron status during experiment process.

4. Conclusion

With the result of two methods, there had a conclusion that liquid membrane method (in combination with oxygen and evacuated replacement) was more effective than hollow fiber module method. Hb could keep more stable and increase oxygen affinity by using liquid membrane method in oxygenation experiment. It has a significant preparation for Hb purification.

References


