

The Effect of Time and pH on Hemolysis During Cardiopulmonary Bypass

A. Bansal¹, C. Hampton¹, Z. Badri¹, K. Savage¹, J. R. Pohlmann¹, J. M. Toomasian¹ and K. E. Cook¹

¹Department of Surgery, University of Michigan, Ann Arbor, Michigan

Abstract

Objective: During cardiopulmonary bypass (CPB), the breakage of red blood cell membranes (hemolysis) and activation of humoral components (i.e. enzymes) in the blood can occur. Hemolysis and humoral component activation can lead to multiple post-operative complications (i.e. renal and lung failure). The purpose of this study is to investigate the causes of hemolysis during CPB. We hypothesize that blood damage during CPB is caused primarily by exposure of the blood to air and negative pressure during cardiotomy suction, which is used to collect blood that is lost during the operation. **Methods:** An in-vitro model was used to investigate the role of time and pH on hemolysis during the application of negative pressure and an air-blood interface. In the time experiment, ovine blood was exposed to pressure of -600 mmHg and room air-flow of 50 mL/min at increasing times (1, 5, 10, 15, and 30 min). In the pH experiment, ovine blood was exposed to a negative pressure of 600 mmHg, and 50 mL/min of either 25% CO₂ or room air-flow (approximately 0.038% CO₂) for ten minutes. Twenty-five percent CO₂ was used to mimic normal physiological pH. Citrate and heparin anti-coagulants were used to prevent clotting in both experimental groups. **Results:** Red blood cell lysis increased linearly with the time that the blood was exposed to negative pressure and air. In the pH experiment, there was no significant difference in hemolysis between the room air group and the 25% CO₂ group. **Conclusion:** Hemolysis during CPB is time-dependent when exposed to negative pressure and an air-blood interface. Rapid changes in blood pH do not contribute to this hemolysis.

Introduction

Cardiopulmonary bypass (CPB) is performed nearly one million times annually for cardiovascular surgery. During CPB, hemolysis (red blood cell membrane rupture) and blood component activation are prominent. This can lead to numerous postoperative complications including renal and lung failure. Compared to other forms of extracorporeal life support, CPB introduces the blood to an interface of air and negative pressure through cardiotomy suction. Typically, cardiotomy suction can expose blood in the surgical field to negative pressures ranging from

-50 mmHg to -100 mmHg, but often times the suction becomes occluded decreasing pressures as low as -600 mmHg. Cardiotomy suction involves the process of mixing blood from the surgical field into an oxygenation circuit in order to restore lost blood. This avoids the use of human donors, which is expensive and runs additional transfusion risks. Previous research has shown that a combined negative pressure and air interface, similar to that of cardiotomy suction, can cause elevated levels of hemolysis¹ (Fig. 1). This study was designed to determine the effects of blood pH and time on hemolysis levels during the application of a combined negative pressure and air interface.

Methods

Researchers developed an in-vitro model capable of simulating and controlling conditions observed during cardiotomy suction in the clinical CPB. The model consists of a sealed test chamber with access ports for applying controlled levels of suction, or a controlled air-blood interface, or any combination (Fig. 2).

Ovine blood was filled into a 60 mL syringe containing 6 mL of citrate and 1 mL of heparin (anticoagulants), at latest 48 hours prior to experimentation. Five mL blood aliquots were distributed into experimental and control test tubes. In the time experiment, 5 mL of ovine blood was exposed to a pressure of -600 mmHg and room air-flow of 50 mL/min for varying times: 1, 5, 10, 15, and 30 min (n=10). Control samples (n=10) were open to room-air and unperturbed for the duration of experimentation. In the pH experiment, experimental samples were divided into two groups. In group one (n=4), room-air was used as the bubbling gas. In group two (n=14), 25% CO₂ was used as the bubbling gas in order to mimic physiological pH (7.4 +/- 0.2). In both groups, ovine blood was exposed to a pressure of -600 mmHg, and 50 mL/min of gas flow for ten minutes. The control samples (n=12) were left open to room-air and unperturbed for the duration of experimentation. Blood gases were recorded immediately after each sample was exposed to experimental conditions. All experimental bloodsamples were collected from the test chamber and placed into centrifuge tubes. All samples were spun down in a centrifuge for 10 minutes in order to separate plasma from erythrocytes, leukocytes, and thrombocytes. Plasma was placed into a spectrophotometer to determine plasma free hemoglobin

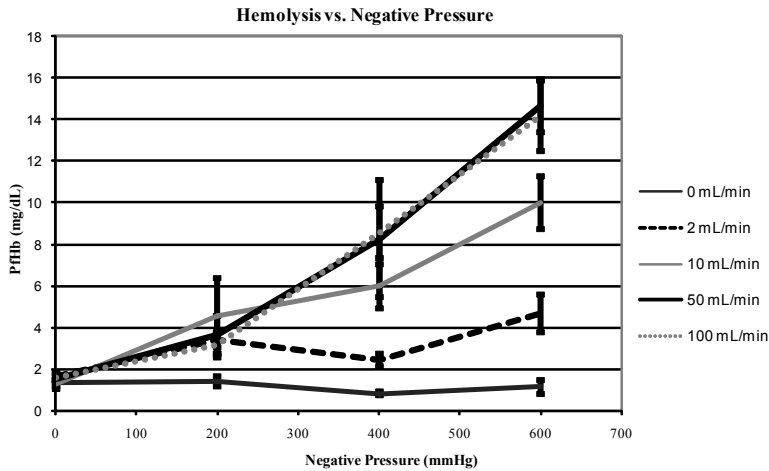


Figure 1: The relationship between hemolysis and negative pressure for 5 different air-flow rates (Pohlmann 2006). There is a synergistic effect of negative pressure and air-flow that causes increasing levels of hemolysis.

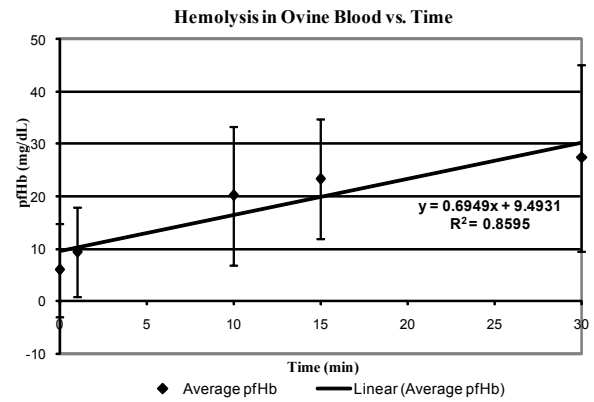


Figure 3: Hemolysis in Ovine Blood vs. Time. Hemolysis levels increase linearly with respect to time. Each point represents the average hemolysis after ten independent trials.

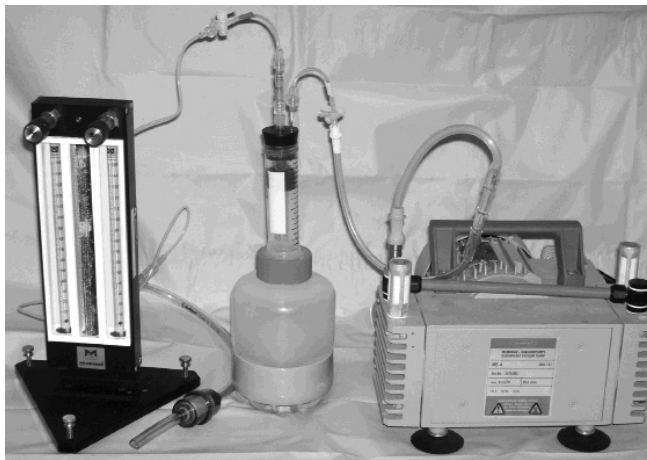


Figure 2: Experimental Design; An air flow meter (left) was used to regulate air flow in to the test chamber (middle). A negative pressure pump (right) was used to create sub-atmospheric pressure conditions within the test chamber.

(pfHb) levels.

Results

Hemolysis levels in ovine blood increased linearly with the amount of time of exposure to an air-negative pressure interface. PfHb ranged from 9.44 mg/dL, after 1 minute, to 27.39 mg/dL, after 30 min. Average pfHb in control samples was 6.00 mg/dL. This is a linear increase of approximately 0.695 mg/dL/min (Fig. 3).

Blood alkalosis (excessive decrease in H⁺ ion) did not appear to contribute to hemolysis levels. The average pH of post-trauma room air trials was 8.279 +/- 0.133, while post-trauma 25% CO₂ samples maintained a pH of 7.269 +/- 0.201 (close to physiological pH). Hemolysis between the 25% CO₂ group and the room air group was not

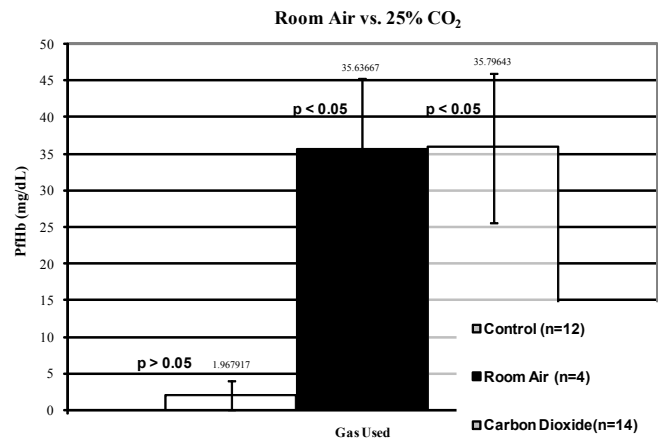


Figure 4: Room Air vs. 25% CO₂; Hemolysis levels between the 25% CO₂ group and the room air group were similar; however, both were significantly higher than control samples.

significantly different ($p > 0.05$). Average pfHb for room air trials (alkalized blood) was 35.69 mg/dL +/- 9.57 mg/dL. Control pfHb (1.96 mg/dL +/- 0.20 mg/dL) was significantly lower than both experimental trials ($p < 0.05$). Average pfHb for 25% CO₂ trials (blood maintained close to physiological pH) was 35.79 mg/dL +/- 10.20 mg/dL (Fig. 4).

Discussion

Hemolysis in ovine blood is a function of negative pressure, air flow, and time. Results demonstrate a linear relationship between the amount of time and the amount of red blood cell lysis; however, there may be limitations to this experimental model. After approximately fifteen

minutes of air-flow, there was a decrease in bubbling due to clot formation. The narrow opening at the base of the flow tube exposed a small portion of the blood to a harsh interface of air-flow that caused the blood to clot within the opening. This occluded the air-flow line and prevented the ovine blood from uniform exposure to air and negative pressure for times above fifteen minutes. A revision of this model to incorporate a wider opening at the base of the air flow tube may help to eliminate clotting and be more representative of cardiectomy suction during CPB.

PfHb was measured as an indicator of blood damage. However, systemic inflammatory response syndrome, common in postoperative CPB patients, is likely caused by leukocyte activation. Further research is required to measure platelet and leukocyte levels under similar conditions. This will provide researchers with a better indication of the mechanisms for blood activation leading to systemic inflammatory response syndrome. Long-term research goals focus on measuring hemolysis, leukocyte activation, and platelet activation on an in-vivo animal model undergoing clinical CPB conditions.

Ovine blood was the model for this study's experiments. Currently, researchers are evaluating changes in human blood with a similar set of experimental conditions.

Conclusion

The combination of air and negative pressure has a synergistic effect on ovine blood that creates significant levels of red cell hemolysis. Hemolysis is linearly proportional to the time that blood is exposed to experimental conditions. In a clinical setting, cardiectomy suction can persist at varying pressures for hours leading to elevated levels of systemic hemolysis and potential leukocyte activation. The time dependence of hemolysis suggests that the clinical use of cardiectomy suction should be avoided or minimized to better prevent damage to red blood cells. The mechanism for hemolysis is likely multi-factorial; however, rapid changes in blood pH do not seem to contribute to this mechanism.

References

1. J. Pohlmann, J. Toomasian, C. Hampton, A. Romeo, R.H. Bartlett, K.E. Cook, *Journal of the Society of Artificial Internal Organs* **52**, 51 (2006).