Validation of Hypoxic Red Blood Cell Storage Procedure and Interrogation of its Vasoregulatory Effects

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BACKGROUND

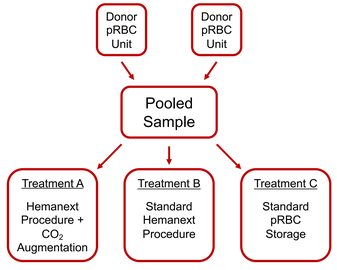
Red blood cells (RBCs) generate and export vasoregulatory mediators as a function of their metabolic context. S- nitrosothiol (SNO) is exported to effect hypoxic vasodilation ensuring tissue oxygenation[1]. The generation and export of adenosine triphosphate (ATP) and SNO also assist in the maintenance of RBC deformability necessary for transit through narrow capillaries and the ability of RBCs to resist adhesion to endothelial cells[2]. Conventional RBC storage leads to the development of storage lesions that compromise RBC quality[3]. Recently, a method of hypoxic RBC storage has been FDA approved, Hemanext, which mitigates oxidative stress (driven by abundant O_2) and preserves 2,3 BPG and to some extent ATP[4]. Hypoxic RBC storage has been shown to provide a protective influence on the appearance of RBC senescence markers (ROS increases, phosphatidylserine exposure, and calcium entry), and favorably influenced outcomes in a rat transfusion model[5].

OBJECTIVE

To elucidate effects of hypoxic pRBC storage on RBC vasomediator export, specifically SNO and ATP.

METHODS

- So far, we have prepared 10 units of hypoxic stored RBCs and 5 control units
- Of the 10 hypoxic stored blood units
  - 5 underwent CO_2 repletion during the deoxygenation reaction process (which otherwise profoundly depletes CO_2 as well as O_2)
  - 5 underwent the standard Hemanext procedure.
- 5 control units were stored under normal storage conditions
- The units were stored and sampled over the course of 6 weeks with supernatant saved for future assays
  - Blood gases were run on all samples at the time of sampling
  - ATP and SNO assays are planned for future analysis



BLOOD GAS DATA

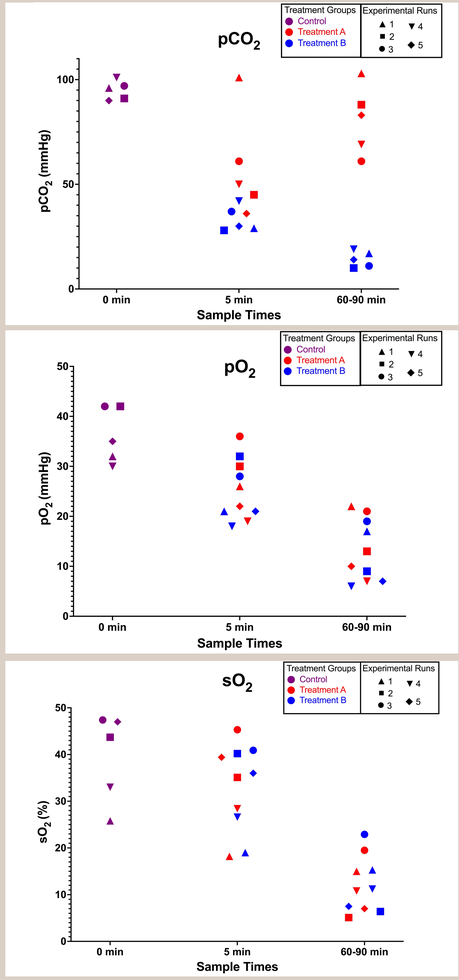


Figure 1
Unit pCO_2 (top) and pO_2 (middle) and sO_2 percentage (bottom) of units throughout hypoxic Hemanext procedure and in control units. Treatment A with CO_2 repletion, Treatment B standard protocol.

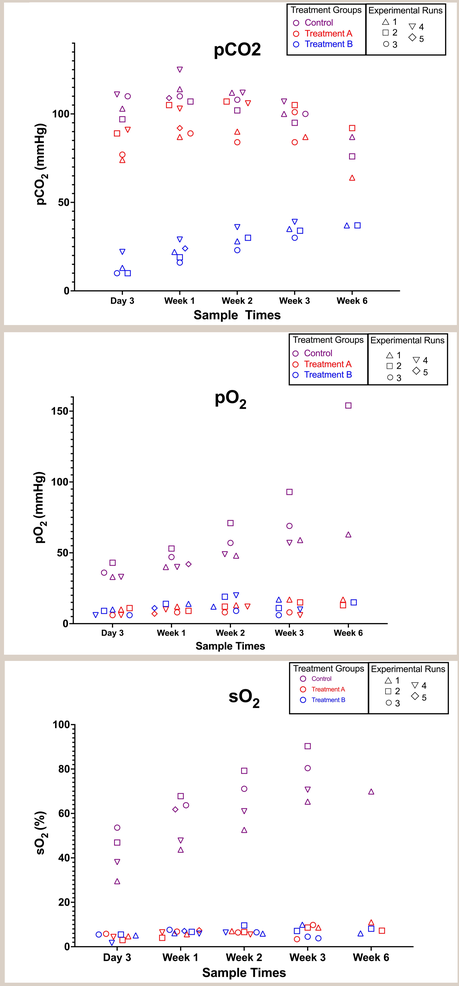


Figure 2
Unit pCO_2 (top) and pO_2 (middle) and sO_2 percentage (bottom) of units during storage following Hemanext procedure. Treatment A with CO_2 repletion, Treatment B standard protocol.

RESULTS

The average sO_2 in the hypoxically stored RBC units was 6.3% and 6.2% for the CO_2 repleted and standard Hemanext procedure respectively, and 61.2% in the control units. The average pCO_2 of the CO_2 repleted group and control group were 90.8 mmHg and 104.2 mmHg, respectively, while standard Hemanext procedure units pCO_2 average was 26.1 mmHg.

CONCLUSIONS

Our study establishes a reliable method of hypoxic blood storage and sampling technique for the assessment of RBC ATP and SNO export throughout the storage duration. Currently, vasomediator assays are underway to complete the study to understand how this novel RBC storage approach might influence responses to RBC transfusion.

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