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TITLE: CO₂ Effects During Anaerobic Storage of RBC

AUTHORS (LAST NAME, FIRST NAME): Dumont, Larry J.^{1, 3}; Baker, Sharry³; Calcagni, Kristin E.³; Housman, Molly L.³; Waters, Susan L.³; Herschel, Louise H.³; Yoshida, Tatsuro²

INSTITUTIONS (ALL): 1. Pathology, Dartmouth Medical School, Lebanon, NH, United States.

2. New Health Sciences, Bethesda, MD, United States.

3. Pathology, Dartmouth-Hitchcock, Lebanon, NH, United States.

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ABSTRACT BODY:

Background: Previous studies indicated that anaerobic RBC storage reduces oxidative damage, maintains ATP & 2,3-diphosphoglycerate (DPG) levels and has superior 24 hr recovery at 6 weeks compared to standard storage. Removal of CO₂ during O₂ depletion by gas exchange may have affected RBC during anaerobic storage. As a preliminary investigation prior to the design of the final O₂ depletion device that doesn't require gas purging, we sought to determine the consequences of CO₂ removal on anaerobic storage of RBC.

Methods: This is a matched 3 arm study: control, O₂&CO₂ depleted with Ar, O₂ depleted 95%Ar/5%CO₂. Whole blood was collected into CP2D (Pall), centrifuged 2KxG 3min, plasma removed, and additive solution AS-3 (Nutricel, Pall), or experimental OFAS3 added. The unit was evenly divided into 3 600mL bags. 2 bags were gas exchanged x7 with Ar or Ar/CO₂, transferred to 150mL PVC bags and stored 1-6°C in anaerobic cylinders with Ar/H₂ or Ar/H₂/CO₂. One control bag was treated in the same manner without a gas exchange and stored 1-6°C in ambient air. Bags were sampled weekly for up to 9 weeks for a panel of in vitro tests including intra- and extra-cellular pH (pHi, pHe).

Results: Twelve subjects were entered into the study, 8 have completed 9 weeks storage. Purging with Ar results in alkalization of the RBC and upregulation of glycolysis. pH and lactate of Ar/CO₂ purged RBC were equivalent to aerobically stored controls (p>0.5, days 0-21). ATP levels are higher in Ar/CO₂ (p<0.0001). DPG was maintained beyond 2 wk in the Ar purged arm only (p<0.0001). Surprisingly, DPG was lost at the same rate in both control and Ar/CO₂ arms (p=0.6). Hemolysis was low in all arms, but may be influenced by the weekly mixing.

Conclusion: The addition of 5%CO₂ to the purging gas prevented CO₂ loss with an equivalent starting pHi and pHe to control bags. Maintenance of ATP in the Ar/CO₂ arm demonstrate ATP production is not solely a function of the pH effect on glycolysis. CO₂ in anaerobic storage prevented the maintenance of DPG, and DPG appears to be pH dependent. Additional investigation is warranted to understand the mechanisms of these metabolic dynamics. CO₂ as well as O₂ depletion provides metabolic advantage for stored RBC.

Biochemical profile of stored RBC

Arm	Day	pHi(22°C)	ATP ($\mu\text{mol/gHb}$)	2,3-DPG ($\mu\text{mol/gHb}$)	Lactate (mmol/gHb)	Hemolysis (%)
Control	0	7.11 \pm 0.04	4.1 \pm 0.7	12.2 \pm 1.8	0.01 \pm 0	0.20 \pm 0.04
	21	6.77 \pm 0.05	4.3 \pm 1.0	0.2 \pm 0.2	0.11 \pm 0.02	0.20 \pm 0.06
	42	6.56 \pm 0.06	3.1 \pm 0.8	0.2 \pm 0.1	0.16 \pm 0.02	0.30 \pm 0.10
	63	6.44 \pm 0.07	2.1 \pm 0.6	0.3 \pm 0.2	0.2 \pm 0.02	0.53 \pm 0.20
Ar/CO ₂	0	7.14 \pm 0.03	4.6 \pm 0.7	12.2 \pm 1.8	0.02 \pm 0	0.17 \pm 0.04
	21	6.76 \pm 0.04	5.5 \pm 1.3	0.2 \pm 0.2	0.1 \pm 0.02	0.19 \pm 0.08
	42	6.58 \pm 0.05	4.0 \pm 1.3	0.1 \pm 0.0	0.15 \pm 0.03	0.32 \pm 0.11
	63	6.51 \pm 0.06	2.4 \pm 1.0	0.1 \pm 0.0	0.19 \pm 0.03	0.61 \pm 0.26
Ar	0	7.38 \pm 0.06	4.6 \pm 0.8	14.3 \pm 1.6	0.02 \pm 0	0.18 \pm 0.05
	21	6.67 \pm 0.04	4.7 \pm 0.9	6.2 \pm 3.0	0.16 \pm 0.02	0.19 \pm 0.04
	42	6.42 \pm 0.06	3.3 \pm 0.9	0.4 \pm 0.2	0.21 \pm 0.02	0.28 \pm 0.08
	63	6.31 \pm 0.09	1.8 \pm 0.9	0.4 \pm 0.2	0.24 \pm 0.02	0.64 \pm 0.35

mean \pm sd