DISCOVERY BIOLOGY

Griffith UNIVERSITY

In vitro susceptibility of K13 mutant *Plasmodium falciparum* isolates to DHA at early ring stage in hyperoxic vs normoxic conditions, and the impact of serum.

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CONCLUSION

DHA resistant K13 mutant *Plasmodium falciparum* acquires enhanced susceptibility to DHA under hyperoxic (21% O_2) *in vitro* culturing conditions. This phenotype is due to adaptation of the parasite during a single development and proliferation cycle from early ring stage to newly invaded RBC. This altered phenotype is **NOT** observed when serum is replaced completely with Albumax II, a serum replacement commonly used for *in vitro* culture of *P. falciparum*.

INTRODUCTION

- *Pf*13 is a biological marker of artemisinin resistance associated with ACT clinical treatment failure
- *In vitro*, the *Pf*K13 mutant F32-ART5 was generated under 21% O_2 conditions (hyperoxic), with 20% serum employed after each drug challenge.
- Other attempts at *in vitro* generation of *Pf*K13 mutations by escalating drug dosing in 5% O_2 (normoxic) protocols have not identified K13 mutations.
- Questions raised 1) does oxygen influence *Pf* susceptibility to artemisinins before, during and after drug exposure? and 2) does serum have a impact on artemisinin resistance.

METHODS

- PFK13 containing *Pf* (MRA-1241) was cultured in 5% CO_2 and either 5% or 21% O_2.
- Cultures incubated in alternative conditions at different asexual stages of growth were then evaluated for parasite survival using the ring stage survival assay (RSA).
- Comparison of effect of media containing serum plus Albumax II, and Albumax II alone was made throughout the RSA protocol.

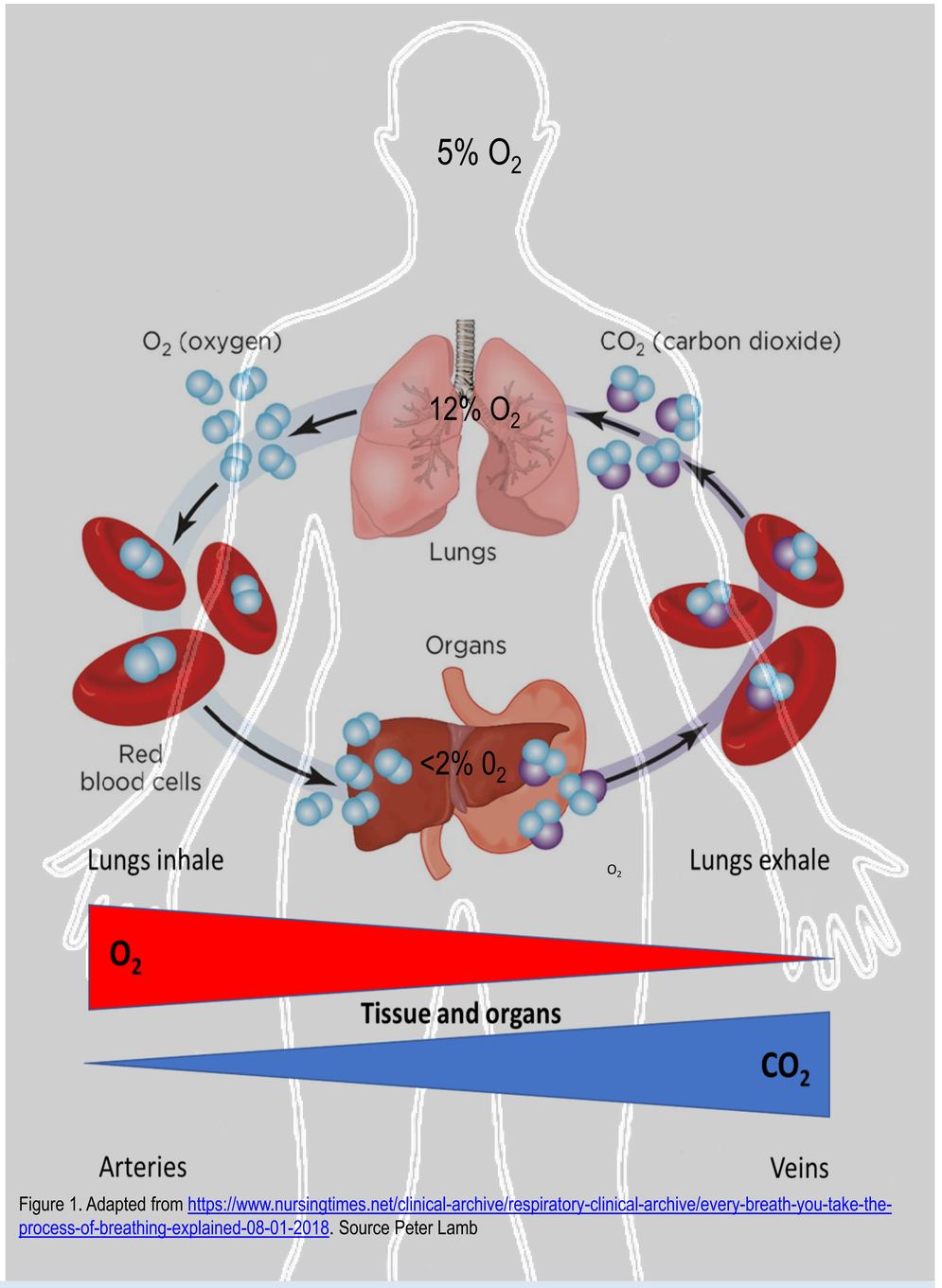

Figure 1. Adapted from https://www.nursingtimes.net/clinical-archive/respiratory-clinical-archive/every-breath-you-take-the-process-of-breathing-explained-08-01-2018. Source Peter Lamb

Figure 1. The human respiratory system and variances in both O_2 and CO_2 throughout the body. When blood passes through the lungs from the veins, CO_2 is expelled through an exhaled breath and O_2 inhaled. RBCs transport oxygen from the lung capillaries to all areas of the body exchanging O_2 for energy production, with CO_2 as metabolic waste. The levels of O_2 and CO_2, unlike a standard laboratory incubator, are highly variable in both concentration and ratio to one another. The intra-erythrocytic form of the *Pf* parasite therefore encounters a vast array of gaseous conditions throughout an infection. However, RBCs also monitor and balance their oxygen binding to compensate for these varied environmental changes in oxygen concentrations.

ACKNOWLEDGMENTS

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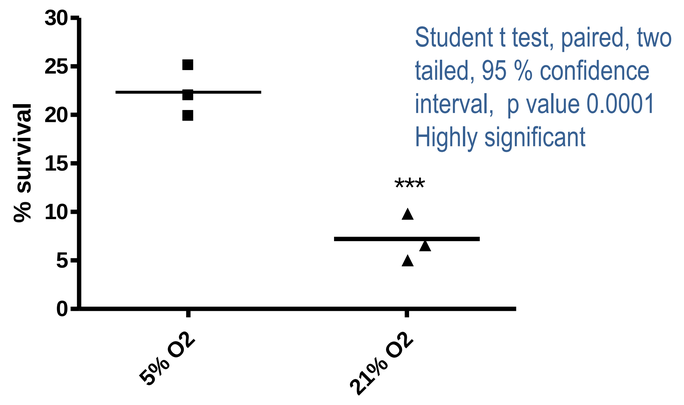
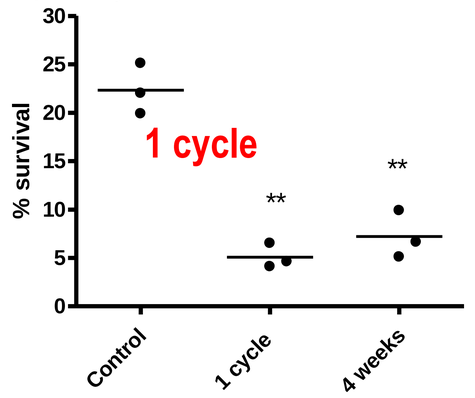
RESULTS

1) Long term culture of MRA-1241 incubated in 5% O_2 vs 21% O_2 - effect on RSA.

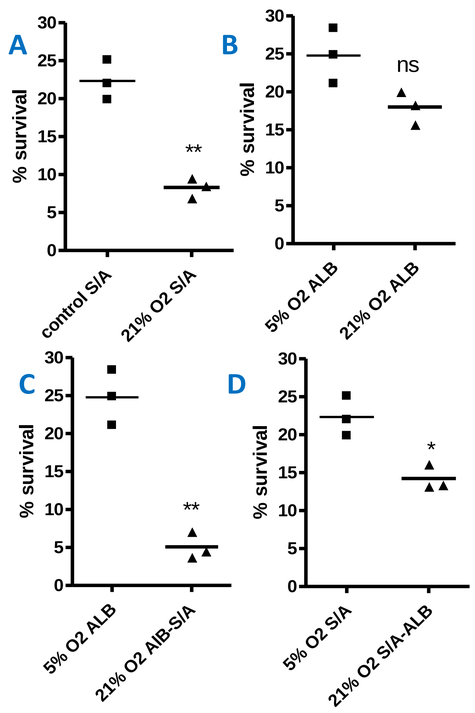


Student t test, paired, two tailed, 95 % confidence interval, p value 0.0001 Highly significant

2) Time in high O_2 conditions for enhanced parasite susceptibility to DHA



3) Serum effect on hyperoxic phenotype



The enhanced susceptibility of *Pf* to DHA in hyperoxic conditions (21% O_2) (**A**) is not obtained when Albumax II only medium is used (**B**). Changing from Albumax to serum containing medium at DHA treatment (**C**), recovers the hyperoxic phenotype indicating a role for serum in DHA activity at elevated O_2 levels. Albumax only media at DHA treatment partially reduces the hyperoxic phenotype (**D**) for parasites cultured longterm in serum.