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Permeability and diffusivity of nitric oxide in human plasma and red cells

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ABSTRACT

A simple diffusion cell was made to measure the permeability and diffusivity of Nitric Oxide in human plasma and red cells. Nitric oxide was passed through the cell containing plasma or nitrited red cells enclosed by silicone membranes. Steady state permeability ($\alpha_{NO}D_{NO}$) was calculated from the cell dimensions and from the NO bulk flow entering and leaving the cell. The diffusion coefficient (D_{NO}) was calculated in three ways: (i) by dividing the steady state permeability by published values for solubility (α_{NO}) in water at 26 °C and 37 °C (ii) by a numerical method and (iii) by an analytical method. Mean steady state permeability (95% confidence intervals) were plasma (26 °C) 5.57×10^{-11} (2.35×10^{-11}–1.32×10^{-10}) and (37 °C) 5.48×10^{-11} (2.13×10^{-11}–1.41×10^{-10}) mol cm^{-1} s^{-1} atm^{-1} and red cells (26 °C) 6.74×10^{-12} (1.29×10^{-12}–3.53×10^{-11}) and (37 °C) 3.93×10^{-11} (1.39×10^{-11}–$1.11.10^{-10}$) mol cm^{-1} s^{-1} atm^{-1}. Median Diffusion Coefficients (D_{NO}) for plasma at 37 °C ranged from 3–3.36×10^{-5} $cm^2 s^{-1}$ and red cells 2.41–2.94×10^{-5} $cm^2 s^{-1}$ depending on the method used. These values may be used for modelling NO transport *in vivo* in the human lung and capillary. Parameters used for modelling *in vivo* should be measured at 37 °C.

1. Introduction

The lung diffusing capacity for nitric oxide ((DL_{NO})) reflects alveolar capillary membrane and blood conductance [1–3]. Our group had previously tried to identify whether the blood conductance (or rather its reciprocal, resistance) was due to the plasma, the red cell membrane or the red cell interior. We altered each resistance in turn in a membrane oxygenator model [4]. Altering the resistance of the red cell interior, but not the plasma or membrane, altered conductance (D_{NO}). However others [Olson JS, Kim-Shapiro D personal communications] have pointed out that our method of altering plasma conductance by increasing viscosity using high molecular weight dextrans, may be ineffective as NO may diffuse around these large polymer molecules in a liquid [5].

Here plasma and red cell interior diffusivity were directly compared by measuring permeability $\alpha_{NO}D_{NO}$ (Krogh's "Diffusion Coefficient" where α_{NO} is gas solubility) and Fick's Diffusivity (D_{NO}) using a simple diffusion cell.

Nobody has previously directly measured diffusivity in plasma or red cell interior. There are several measurements made on water [6–8] and physiological fluids [6,9], generally at room temperature. There are three direct measurements of membrane diffusivity [9–11] and two within tissue [12,13]. Most groups modelling blood diffusion of NO have assumed plasma the same as water and red cell interior identical to smooth muscle or they have extrapolated from oxygen diffusion values [14–17].

2. Methods

2.1. Diffusion cell

A cell was constructed from readily available materials [6] replicating Zacharia and Deen's original design (Fig. 1) consisting of two plates made from three laminated layers of 0.04 cm polystyrene sheeting (Homebase, Huntingdon, PE29 6DA, UK) with concentric apertures shaped to Zacharia and Deen's dimensions (see Fig. 1) with inlet ports made from 3/32" (0.24 cm) alloy tubing (Inwood Model Supplies, Huntingdon PE29 6 EB, UK.) The plates were separated by a 0.4 cm thick and 0.51 cm and 0.32 cm inside and outside diameters respectively neoprene gasket (RAM Gasket solutions, Redruth,UK, TR15 1SS) and 0.025 cm Silastic Membranes (Dow Corning, Michigan USA) sandwiched between the plates and the gasket. The silastic membranes

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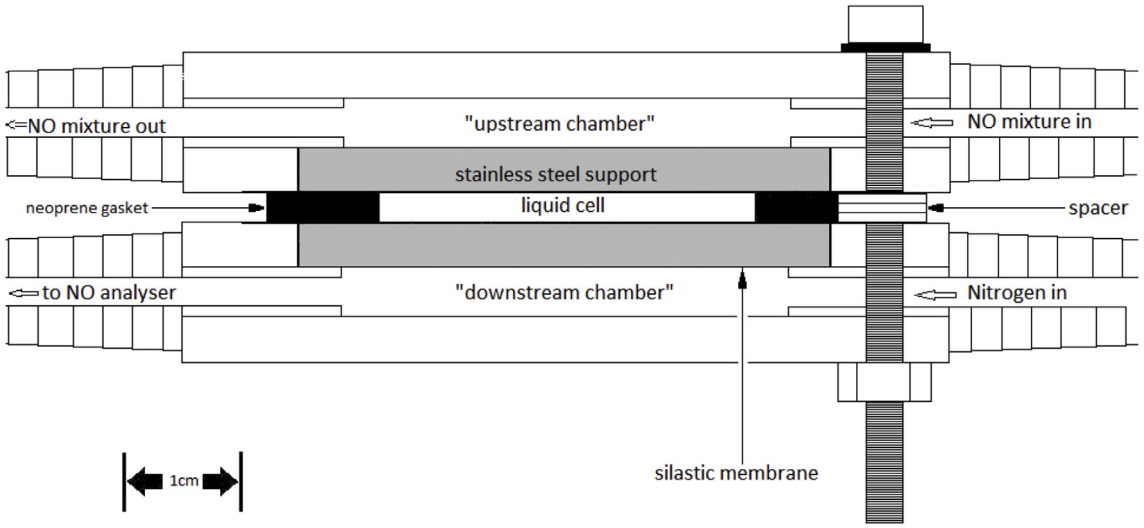

Fig. 1. Cross sectional diagram of diffusion cell.
Only one of the M4 bolts is shown for clarity.

were kept flat by stainless steel support screens (Millipore Corp, Billerica, Massachusetts, USA). For measurements on the empty cell three layers of silicone membrane were sandwiched between the Teflon gaskets and the downstream steel support only. The assembly was secured by four M4 bolts. Three M4 washers made the thickness of the liquid layer 0.27 cm.

2.2. Calibration/analyser

For daily calibration the three layers of silicone membrane were replaced by a 0.018" (0.0457 cm) circular stainless steel baffle (K&S Engineering 6917 W 59th St Chicago IL 60638, USA) and the downstream chamber flushed with 110 ppb NO in nitrogen (manufacturer's certificate of analysis, BOC Special products www.boconline.co.uk/en/products-and-supply/speciality-gas/index.html.This concentration was chosen to cover the range of NO concentrations at the detector when testing membrane alone, plasma and red cell. Once a stable signal was achieved (typically after 15 min) the average of three readings was taken.

2.3. Randomisation

One run was performed each day. Experiments were performed one week apart in three blocks: cell containing liquid at room temperature, empty cell and cell containing liquid at 37 °C. Within each "liquid" block four replicates of plasma and four of red cells were performed. The order within each of the "liquid" blocks was randomised.

2.4. Procedure

To remove oxygen (which could react with NO) the upstream and downstream chambers of the cells were flushed with nitrogen via a rotameter flowmeter (150 MM for nitrogen, Omega Engineering, Irlam, Manchester M44 5BD UK) for an hour at a rate of 250 ml/min which Zacharia and Deen found adequate to flush the cell in their simulation [6]. The gas flow to the upstream chamber was then switched to 1000 ppm NO in nitrogen (for the empty cell) or to 1% NO in nitrogen (for plasma and red cells) (NO in nitrogen, gases manufacturer's certificate of analysis, BOC Special products specialproducts@boc.com). The NO was not passed through soda lime to remove higher oxides of nitrogen as this process caused loss of signal [1]. The downstream chamber was kept flushed with nitrogen at a flow rate of 250 mL/min.

The NO concentration of the downstream chamber was sampled at half minute intervals for 60 min using a chemiluminescent analyser (Logan Research LR2000, Rochester, ME2 2NP, UK).

All connections were made with PTFE (Teflon)Tubing (0.6 cm inside, 0.8 cm outside) (Hilltop Products, Golborne WA3 3PY, UK) to avoid reaction with NO. Because this tubing is rigid, connections to the cell, flowmeter, and cylinders were fashioned from 3/8" (0.952 cm) PVC perfusion tubing (www.livanova.sorin.com). These connections were as short as possible.

2.5. Preparation of blood/plasma

20 mL of venous blood was drawn from a healthy volunteer into two EDTA tubes. For plasma the tubes were immediately centrifuged for 5 min at 3000 rpm and the supernatant plasma collected and pipetted into the diffusion cell as described below. For concentrated red cells the supernatant plasma was discarded. To preserve cellular integrity the cells were therefore re-suspended in a volume of phosphate buffer saline (PBS) (Sigma Aldrich, Gillingham, SP8 4XT, UK.) (equal to the plasma removed) to which 2 mL of 3% sodium nitrite (Ipswich Hospital Pharmacy Department, Ipswich IP4 5PD, UK) had been added. After standing for 10 min the sample was re-centrifuged and the supernatant was discarded. The stoichiometric and kinetic basis for this procedure is explained in Appendix A. The nitrited cell concentrate was pipetted into the diffusion cell as described below.

2.6. Loading the cell

To add liquid to the cell both sides of the Teflon gasket were coated with a thin layer of high vacuum grease (Vacuum Lubricant, Dow Corning, and Global Industrial Verona NJ 07044, USA). A silicone membrane was placed onto the laboratory bench and the greased gasket firmly pressed onto it. Approximately 2.2 mLs of liquid (plasma or red cell concentrate) were then carefully pipetted onto the membrane until the liquid surface just reached the top of the gasket. The second membrane was gently pressed onto the gasket, taking care to expel all air bubbles. The assembled cell was then placed on top of the stainless steel spacers and the top layer of the cell screwed down by tightening the four M4 nuts.

2.7. Temperature

Experiments were carried out at laboratory temperature (average 26 °C) or at 37 °C.For the former the cell was mounted on a wooden