Respiratory Physiology & Neurobiology 246 (2017) 53–58

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Air blood barrier phenotype correlates with alveolo-capillary O_2 equilibration in hypobaric hypoxia

Egidio Beretta[a,*], Francesca Lanfranconi[a], Gabriele Simone Grasso[a], Manuela Bartesaghi[a], Hailu Kinfu Alemayehu[b], Lorenza Pratali[c], Bruna Catuzzo[d], Guido Giardini[d], Giuseppe Miserocchi[a]

[a] Ambulatorio di Fisiologia Clinica e dello Sport, Scuola di Specializzazione in Medicina dello Sport, Università di Milano-Bicocca. Via Cadore, 48 20900 Monza, Italy
[b] Dipartimento di Scienze Mediche e Biologiche, Università degli Studi di Udine, Piazzale M. Kolbe 4, 33100 Udine, Italy
[c] Istituto di Fisiologia Clinica – CNR, Via Moruzzi 1 Pisa, Italy
[d] Centro di Medicina di Montagna, Ospedale Regionale "U. Parini", Aosta, Italy

ARTICLE INFO

Keywords:
High altitude pulmonary edema
DLCO
Diffusion subcomponents
Lung interstitial edema
Diffusion limitation
oxygen diffusion-transport

ABSTRACT

The O_2 diffusion limitation across the air blood barrier (DO_2 and subcomponents *Dm* and *Vc*) was evaluated in 17 healthy participants exposed to hypobaric hypoxia (HA, 3840m, P_IO_2 ~90 mmHg). A 10% decrease in alveolar volume (*VA*) in all participants suggested the development of sub-clinical interstitial lung edema. In > 80% of participants DO_2/VA increased, reflecting an individual strategy to cope with the hypoxia stimulus by re-modulating *Vc* or *Dm*. Opposite changes in *Dm*/*Vc* ratio were observed and participants decreasing *Vc* showed reduced alveolar blood capillary transit time. The interplay between diffusion and perfusion (cardiac output) was estimated in order to investigate the individual adaptive response to hypoxia. It appears remarkable that despite individual differences in the adaptive response to HA, diffusion limitation did not exceed ~11% of the alveolar-venous PO_2 gradient, revealing an admirable functional design of the air-blood barrier to defend the O_2 diffusion/perfusion function when facing hypobaric hypoxia corresponding to 50 mmHg decreased P_AO_2.

1. Introduction

Exposure to hypobaric hypoxia on reaching high altitudes leaves as open question the potential role of diffusion limitation to affect the alveolar-capillary PO_2 gradient. A new impetus to answer this question was given by the development of an individual-based method allowing to effectively compare the lung diffusion capacity at sea level and at high altitude, as in the latter condition lung diffusion is known to be affected by the greater hemoglobin affinity of CO (the tracer gas used to estimate lung diffusion capacity) (Beretta et al., 2017). The present paper is aimed at providing new knowledge concerning the adaptive responses of the air-blood barrier (ABB) to hypobaric hypoxia (3840m), that is known to represent a strong edemagenic condition (Miserocchi et al., 2001) that *per se* may affect the lung diffusion capacity (Bartesaghi et al., 2014). We attempted to characterize the adaptive hypoxia response based on the individual ABB phenotype, as defined by its diffusive/perfusive properties namely *Dm*, the air-blood membrane diffusion, and *Vc*, an estimate of the extension of the alveolar capillary network. The impact of diffusion limitation was estimated by correlating the individual diffusive/perfusive properties with the overall diffusive/perfusive capacity ratio of ABB based on the non-invasive model of Piiper and Scheid (1981) allowing to define the kinetics of the alveolo-venous equilibration process.

2. Material and Methods

2.1. Participants

Data were obtained from 17 healthy participants (12 males, 5 females), average age 36.4 ± 8.2, who regularly practiced mountaineering and/or mountain hiking. All participants were no smokers or mild smokers (less than 4 cigarettes/day) and their spirometric parameters were above 90% of predicted values. The research project was approved by the ethical committee of University of Milano Bicocca and was conducted in accordance with the Helsinki Declaration on human to assure ethical standards were being met. Participants were instructed about the experimental procedure and related discomfort, as well as of the risks of acute exposure to hypoxia, and signed an informed consent.

All measurements were performed at sea level (SL, Monza Italy, 170m, P_IO_2 157 mmHg) and at high altitude (HA, Aiguille du Midi,

* Corresponding author. Beretta Egidio, Dipartimento di Medicina e Chirurgia, Università di Milano Bicocca, Via Cadore, 48 20900 Monza, Italy.
E-mail address: egidio.beretta@unimib.it (E. Beretta).

http://dx.doi.org/10.1016/j.resp.2017.08.006
Received 6 June 2017; Received in revised form 2 August 2017; Accepted 4 August 2017
Available online 08 August 2017
1569-9048/

3840m, P_IO_2 90 mmHg). Participants spent the night of the eve at SL; the following morning they reached the laboratory at HA by cable car (about 30 minutes) and measurements were done after 4 hours from reaching the laboratory at HA.

2.2. Diffusion measurement

Measurement of *DLCO* and subcomponents were performed at SL and at HA according to standardized procedures at rest, in sitting position at total lung capacity (TLC) by single breath method (QUARK PFT, Cosmed, Roma, Italy). Participants inspired 3 gas mixtures containing 0.3% CH_4 (tracer to measure lung volume, *VA*), 0.3% CO and 20, 40 and 60% O_2, respectively. Each maneuver was performed at least 6 min after the previous one. *Dm* and *Vc* were determined from the experimentally derived linear regression as defined by (Roughton and Forster, 1957):

$$\frac{1}{DLCO} = \frac{1}{Dm} + \frac{1}{\theta \cdot [\mathrm{Hb}] \cdot Vc} \quad (1)$$

where θ is the binding rate of CO with Hb, [Hb] is the ratio between individual hemoglobin concentrations over reference values of hemoglobin concentration for men and women, $\frac{1}{Vc}$ is the slope of the relationship and $\frac{1}{Dm}$ the intercept. We chose a $1/\theta$ value as defined (Forster, 1987) according to the equation:

$$\frac{1}{\theta} = 0.75 + (0.0057 \cdot P_A O_2) \quad (2)$$

where P_AO_2 is assumed equal to the measured end tidal O_2 pressure ($PetO_2$).

DLCO values measured at HA were adjusted according to a recently developed method that accounts for the inter-individual differences of the effect of hypoxia exposure on diffusion subcomponents (Beretta et al., 2017); this approach allows maintaining on numerical basis the validity of Eq. (1). Blood samples were taken in resting conditions at SL to determine Hb concentration and hematocrit. Data were standardized to Hb concentration of 14.6 g/dl in men and 13.4 g/dl in women. DO_2 was derived as 1.23·*DLCO* measured with 20% O_2.

2.3. Echocardiography

Standard 2D echocardiography was performed at rest in supine position using a portable echo machine with a 2.5-3.5 MHz cardiac probe (Vivid I, General Electric Healthcare Clinical System) by a single experienced cardiologist, both at SL and HA. Care was taken to ensure that the position of the participants and the transducer were similar in all examinations. Stroke volume was obtained from apical 4 chamber view. Cardiac output ($\dot{Q}$) was measured multiplying left ventricle outflow tract time-velocity integral, measured using pulse wave Doppler, by its cross-sectional area and heart rate (Lang et al., 2015). Systolic pulmonary arterial pressure (*PAPs*) was estimated from the peak velocity of the tricuspid regurgitation jet by continuous flow Doppler and the systolic right atrium (RA) pressure estimated from the inferior vena cava diameter and its respiratory excursion (0–15 mmHg) using the formula: PAPs = 4 V^2 + RA pressure (Yock and Popp, 1984). Pulmonary vascular resistance (*PVR*) was estimated from the ratio of peak tricuspidal velocity (m/s) to the right ventricular outflow tract velocity-time integral, obtained by placing a 1–2 mm pulsed wave Doppler sample volume in the proximal right ventricular outflow tract, just within the pulmonary valve in the para-sternal short-axis view (Wood unit, Abbas et al., 2003). This relationship was considered valid for *PVR* Wood units < 8 (Rajagopalan et al., 2009).

2.4. Ventilatory and physiologic parameters

Pulmonary ventilation ($\dot{V}E$, in BTPS) and end tidal O_2 and CO_2 partial pressure ($PetO_2$, $PetCO_2$), were determined by a portable metabolic cart (K4b2, Cosmed, Roma, Italy). Heart rate (HR) was determined from a 12-lead electrocardiographic signal interfaced to Sensor Medics metabolic cart. Arterial blood O_2 saturation ($\%SatO_2$) was monitored continuously through oximetry at the finger (RAD 9 Signal Extraction Pulse Oximeter: Masimo Corporation, Irvine − California, USA) and only valid signals were considered checking the quality of the pulse wave signal. The environmental temperature was kept at 18 °C using an air-conditioning system and the current barometric pressure was recorded.

2.5. Estimate of diffusion limitation

Based on the mass conservation principle, Piiper and Scheid (1981) developed a theoretical model considering the equality of the diffusive and convective flux of O_2 when blood flows along the alveolar gas-exchanging unit. According to the model, the degree of diffusion limitation (*Ldiff*) resulting from the alveolar-capillary equilibration at the arterial end of the capillary can be defined as:

$$Ldiff = \frac{P_A - P_a}{P_A - P_{\bar{v}}} = e^{-\frac{DO_2}{\beta \dot{Q}}} \quad (3)$$

being P_A, P_a and $P_{\bar{v}}$ the O_2 partial pressures in the alveolar compartment, in the arterialized blood leaving the capillary and in the mixed venous blood reaching the alveoli, respectively; DO_2 is the O_2 diffusive capacity, $\dot{Q}$ is the cardiac output and β is the Hb binding capacity for O_2 (we assumed β values of 0.83 and 2.5 ml L^{-1} $mmHg^{-1}$ for SL and HA, respectively). The ratio $\frac{DO_2}{\beta \dot{Q}}$, defined as "equilibration index", is a pure number as the numerator and denominator have the units of ml min^{-1} $mmHg^{-1}$ and *Ldiff* varies from 0 (perfect equilibration) to 1 (no equilibration, as in case of arteriovenous shunt). By defining the average pulmonary blood capillary transit time (*Tt*) as $Tt = \frac{Vc}{\dot{Q}}$, one can reformulate diffusion limitation as

$$Ldiff = e^{-\frac{DO_2}{\beta} \cdot \frac{Tt}{Vc}}$$

2.6. Statistical analysis

Values were expressed in Tables as median and Interquartile Range (IQR 25th-75th percentile). Other values are reported as mean ± SD. The statistical significance of the difference for paired samples was estimated with Wilcoxon test. Regression and correlation analyses were performed using the least squared residuals method. All statistical analyses were performed by utilizing a commercially available software package (Origin, Origin Lab Corporation).

3. Results

Fig. 1A shows the relationship between *DLCO* measured at HA (adjusted according to Beretta et al., 2017) vs the corresponding SL values. It appears that data essentially scattered around the identity line, with a tendency to decrease at HA only for subjects having the highest value of *DLCO* at SL. Fig. 1B shows that *DLCO/VA* values increased on exposure to HA in 65% of the participants, did not change in 23% and clearly decreased only in 1 participant, the same showing the highest decrease in *DLCO* (Fig. 1A).

Fig. 2 shows the inverse relationship between the relative changes in *Vc* and *Dm* in HA normalized to SL value.

Fig. 3A shows an important difference when considering the relationship between the changes in *Dm/Vc* on exposure to HA *vs* the corresponding changes in $PetO_2$: in group identified as G1 (n = 5, closed circles), the *Dm/Vc* ratio significantly decreased, while the opposite occurred for the other group, identified as G2 (n = 12, open circles). B shows that values of *Dm/Vc* and of $\frac{DO_2}{\beta \dot{Q}}$ have opposite trends in the 2 groups at SL and at HA.

Table 1 reports the average values for *DLCO*, *VA*, *Dm/Vc*, *Vc/VA* and