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Effects of acute anemia and hyperoxia on oxygen distribution

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Abstract

Objective: We investigated the distribution of the partial pressure of oxygen in the tissue of un-anesthetized hamster window chamber model during anemic conditions (a hematocrit reduction of 40%) at breeding room air and normobaric hyperoxia.

Design and methods: Acute hemodilution was induced by exchanging 40% of the animals estimated blood volume (7% of body weight) with dextran 70 kDa (B. Braun, Irvine, CA). Inspired oxygen (FiO₂) concentration was either 21% or 100% at normobaric conditions. Systemic and microvascular hemodynamics were studied at each FiO₂, including blood pressure (BP), heart rate (HR), hematocrit (Hct), hemoglobin (Hb), blood gases, arteriolar and venular diameters and blood flow, and number of capillaries with red blood cells passage (functional capillary density, FCD). High resolution microvascular intravascular and interstitial pO₂ measurements were made using phosphorescence quenching microscopy (PQM).

Results: Hemodilution decreased Hct from 43% to 25%, with no systemic hemodynamic effects, although microvascular blood flow increased. Change in FiO₂ at baseline Hct increases arterial pO₂ with no significant effects on BP and HR, and decreased arteriolar diameter and blood flow. Measurement of interstitial pO₂ by scanning the tissue along set paths without regard to microvascular structures have a Gaussian distribution (mean $\pm$ SD, mmHg): 29.6 $\pm$ 5.7 (43% Hct, FiO₂ 0.21); 32.0 $\pm$ 10.5 (43% Hct, FiO₂ 1.00); 31.12 $\pm$ 8.0 (25% Hct, FiO₂ 0.21); 26.0 $\pm$ 11.3 (25% Hct, FiO₂ 1.00). The decrease of oxygen carrying capacity by 40% reduced pO₂, paradoxically, the increased FiO₂ did not increase the average tissue pO₂ in the window chamber. All changes are statistically significant relative to baseline, $p < 0.05$.

Conclusions: At normal Hct values and during moderate hemodilution hyperoxia-induced vasoconstriction impairs microvascular oxygen transport and tissue oxygenation in the peripheral microcirculation.

1 Introduction

Hyperoxic ventilation (HV) is used clinically as a means to augment tissue oxygenation through the increase of arterial oxygen content in conditions of anemia. Since haemoglobin is saturated with oxygen when arterialized blood leaves the lung, the increase in oxygen delivery attained by hyperoxic ventilation is restricted to the increase in the amount of oxygen dissolved in plasma [1]. It is generally perceived that oxygen supplementation is beneficial, however there is no compelling evidence that it yields a positive clinical effect. This could be the consequence of increased tissue pO₂ due to increased FiO₂ lowering tissue perfusion due to vasoconstriction, as demonstrated in the vasculature of the brain, heart, and limbs. Exposure of the tissue to hyperoxic conditions causes vasoconstriction as

shown by Lindbom and Arfors [2] in the microcirculation of the rabbit tenuissimus muscle where exposure of the preparation to high oxygen content superfusion solution decreased functional capillary density (FCD, number of capillaries with RBC transit per unit volume of tissue). Thorborg et al. [3] showed that mean tissue pO_2 on the surface of skeletal muscle increases when FiO_2 is progressively increased from normal to 0.70, however as the average tissue pO_2 increased, so did the dispersion of the data, i.e., the number of tissue regions with low and high pO_2 . Whalen and Nair [4] showed a fall in deep tissue pO_2 in cat skeletal muscle when the surface of the tissue was exposed to elevated oxygen levels, an effect also found by Duling [5] in the arterioles of the hamster cheek pouch. Experimental studies tend to show that hyperoxia does not provide a uniform benefit for tissue oxygenation [6,7] since vasoconstriction interferes with capillary oxygen delivery when blood oxygen content and pO_2 are increased in the tissue. This may be due to an oversupply of oxygen resulting from the extra oxygen carried by plasma in combination with the oxygen transported by RBCs. This oversupply of oxygen may not be present in conditions of hemodilution when the intrinsic oxygen carrying capacity of blood is diminished. However the actual outcome when combining hemodilution with hyperoxia in terms of oxygen delivery is not entirely predictable because the decrease in intrinsic oxygen carrying capacity due to hemodilution is in part compensated by the increase in cardiac output due to the lowered blood viscosity. The present study was carried out to determine the functional effects of normobaric hyperoxic ventilation (100% O_2 , $FiO_2 = 1.00$) in the microcirculation in unanesthetized hamster window chamber model in conditions of isovolumic hemodilution and determine if vasoconstriction interferes with tissue oxygenation.

2 Materials and Methods

Animal Preparation

Investigations were performed in Golden Syrian hamsters (Simonsen; Gilroy, CA) of 55–65 g body wt size. Animal handling and care was provided in accordance with the procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was approved by the local Animal Subjects committee. The hamster window chamber model is widely used for microvascular studies in the unanesthetized state, and the surgical technique is described in detail elsewhere [8].

Systemic parameters

MAP was tracked continuously during the experimental period, and HR was determined from the pressure trace (Spectramed Pressure Transducer, Biopac; Santa Barbara, CA). Systemic Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes (Readacrit Centrifuge, Clay Adams, Division of Becton and Dickinson; Parsippany, NJ).

Blood chemistry

Arterial blood was sampled from the carotid artery catheter into heparinized capillary tubes and immediately analyzed for PO_2 , PCO_2 , and pH at 37°C (pH/blood gas analyzer, model 248, Bayer). Hemoglobin concentration was measured using a hand-held photometer (B-Hemoglobin; Hemocue, Sweden).

Inclusion criteria

Animals were included in the study if their systemic parameters were 1) heart rate (HR) > 320 beats/min; 2) mean systemic blood pressure (MAP) > 80 mmHg; 3) systemic hematocrit (Hct) > 45%; and 4) systemic arterial PO₂ > 50 mmHg. The microvasculature of the chamber tissue was examined for edema and bleeding. Animals were excluded from the study when signs of trauma were observed.

Functional capillary density

Functional capillaries are defined as capillary segments that have RBC transit in a 30-s period. Observation was done systematically by displacing the microscopic field of view by a field width across the window chamber in 10 to 15 successive steps. The first field was chosen by some distinctive anatomic landmark (i.e., large bifurcation) to easily and quickly reestablish the same fields throughout the experiment.

Arterioles and venules

Arteriolar and venular diameters were measured by the video image shearing method[9]. Arteriolar and venular RBC velocity were measured on-line by using the photodiode/cross-correlator system[10] (model 102B of Vista Electronics; San Diego, CA). The measured centerline velocity was corrected according to vessel size to obtain the mean RBC velocity (V) [11]. Blood flow was calculated from measured parameters as $Q = V \cdot \pi D/2)^2$ where D is diameter.

Microvascular pO₂ distribution

PO₂ measurements were made using the Pd-phosphorescence quenching method developed by Wilson[12] adapted for microcirculatory studies[13] based on the oxygen-dependent quenching of phosphorescence emitted by albumin-bound metalloporphyrin complex after pulsed light excitation.

Experimental design

The unanesthetized animal was placed in a restraining tube. A plastic tent with an inlet valve connected to a gas tank was placed in the front of the restraining tube. The restraining tube was attached to the stage of an inverted microscope (IMT-2 Olympus; New Hyde Park, NY) equipped with a water immersion $\times 40$ objective (Olympus Wplan, n.a. = 0.7). The tissue image was projected onto a CCD camera (COHU, San Diego 4815-2000) connected to a video cassette recorder (AG-7355, Panasonic) and viewed on a Sony monitor (PVM-1271Q). Sites of investigation were chosen based on their visual acuity. Six arterioles and six venules in each animal were chosen for investigation using visual acuity as the first criterion and second in terms of vessel type within the network. The same sites of study were followed throughout the experiment so that comparisons could be made directly to baseline levels. Baseline systemic parameters, blood gas analysis, vascular diameter, RBC velocity, and FCD measurements commenced at least 30 min after the animal had become accustomed to the restraining tube and to the tent air-flow system. After microvascular and systemic baseline characterization, the inlet gas to the plastic tent was switched to 100% O₂ to induce hyperoxia. After 5 min of exposure, systemic parameters and blood gas analysis

were evaluated, as well as FCD. The Pd-porphyrin dye was injected over 1 min intravenously and allowed 10 min to distribute. Intravascular oxygen tension in the vessels chosen for study was measured along with vessel diameter and RBC velocity. Finally, blood gas analysis was repeated to ensure that the induced oxygen level had been maintained during the observation period.

Cardiac Output measurements

Cardiac output was measured by a modified thermodilution technique [14]. Measurements were done under normoxic conditions and hyperoxic conditions after an adaptation period of 5 min.

Data analysis

Comparisons were made with the Mann-Whitney rank sum test (GraphPad Prism 4). Changes are considered statistically significant if $P < 0.05$. Calculations that are not directly measurable in our model are based on mean values of measured parameters.

3 Results

The microvascular study was performed using 20 animals. All animals entered the study completed the experimental protocol. All assigned animals tolerated the hemodilution protocol without visible signs of discomfort. The animals were assigned randomly to the experimental groups: 43% Hct, FiO₂ 0.21 (n=5), 43% Hct, FiO₂ 1.00 (n=5), 25% Hct, FiO₂ 0.21 (n=5) and 25% Hct, FiO₂ 1.00 (n=5). Additional animals were used for the separate cardiac output measurements. Systemic conditions of the different groups are given in Table 1. These results show that inspired gas (FiO₂ 1.00) did not statistically modify blood pressure, HR, Hb, Hct, pH, pCO₂, BE and FCD. However, it significantly changed cardiac output; the cardiac output (CO) was reduced to 0.71 for 43% Hct by the increase of FiO₂ to 1.00, hemodilution increased CO to 1.36 for 25% Hct FiO₂ 0.21 and decreased to 0.94 for 25% Hct, FiO₂ 1.00 relative to baseline. FiO₂ of 1.00 caused a significant increase in arterial PO₂ of 620% increase at 43% Hct and 700% increase at 25% Hct. Animals were able to maintain their blood pH and PCO₂. The changes in arteriolar and venular diameters are shown Fig 1; changes were not significantly different from baseline. Fig 2 shows the change of the RBC flow velocity in arterioles and venules following FiO₂ 1.00. Inhalation of high oxygen levels increased flow velocity at normal conditions and decreased flow velocity at Hct 25%. The diameter and RBC velocity data were used to compute microvascular blood flow in the vessels resulting from this calculation. Arteriolar and venular flow after exposure to hyperoxia was increased at normal Hct and decreased at Hct 25%. The tissue pO₂ measured by scanning the tissue along set paths without regard to microvascular structures have a Gaussian distribution (mean $\pm$ SD, mmHg): 29.6 $\pm$ 5.7 (43% Hct, FiO₂ 0.21); 32.0 $\pm$ 10.5 (43% Hct, FiO₂ 1.00); 31.12 $\pm$ 8.0 (25% Hct, FiO₂ 0.21); 26.0 $\pm$ 11.3 (25% Hct, FiO₂ 1.00) (Fig3). All changes are statistically significant relative to baseline, $p < 0.05$. Oxygen tension in the microvascular network in hyperoxic and normal conditions was 36.4 $\pm$ 3.8 (43% Hct, FiO₂ 0.21); 45.0 $\pm$ 7.6 (43% Hct, FiO₂ 1.00); 41.3 $\pm$ 8.5 (25% Hct, FiO₂ 0.21); 51.2 $\pm$ 14.2 (25% Hct, FiO₂ 1.00) in arterioles and 27.3 $\pm$ 4.9 (43% Hct, FiO₂ 0.21); 35.5 $\pm$ 10.7 (43% Hct, FiO₂ 1.00); 26.3 $\pm$ 9.8 (43% Hct, FiO₂ 0.21); 23.1 $\pm$ 8.1 (25% Hct, FiO₂ 1.00) in venules.

Oxygen tension levels in the microvasculature during hyperoxia were statistically different from the corresponding order in normoxia. Table 2 shows the oxygen delivery and consumption in the microcirculation.

4 Discussion

The principal finding of the study was that HV with 100% oxygen has a limited effect on microvascular PO_2 at physiological hematocrit but leads to diminution of oxygen tension in the tissue and a decrease of blood flow velocity after moderate hemodilution. HV increases the amount of oxygen physically dissolved in plasma and by that CaO_2 (arterial oxygen content). Although at physiologic hemoglobin concentrations the percent contribution of plasma oxygen to VO_2 (oxygen consumption) may be considered negligible (approximately 5%), the situation changes after moderate acute normovolemic hemodilution; because of the loss of erythrocytes as oxygen transporters, the percent contribution of physically dissolved oxygen to VO_2 increases to 47% (hemoglobin 7 g/dl) [1]. In the hemodilution group the vasodilatation due to anemia seems to be compensated by the vasoconstrictive component of HV, therefore we do not see any significant difference in vessel diameters between the groups. At physiological Hct blood flow velocity was significantly increased by HV, while it was decreased at anemic conditions, which might be due to the loss of erythrocytes which can act like an oxygen puffer in order to protect the tissue of harm by HV, so that the organism reacts with a reduction of blood flow velocity as compensating mechanism. Moreover, hyperoxia initiated after moderate hemodilution partially reversed the compensatory mechanisms for moderate anemia where blood flow normally increases and created a margin of safety for tissue oxygenation and organ function. However, the oxygen frequency distribution in the hyperventilated 25% Hct group in our study was significantly lower comparing to the other groups, which could be due to lower blood flow velocity.

There are studies that show a positive effect of hyperoxia, i.e. Van Bommel et al [15] reported that hyperoxygenation initiated during extreme anemia increased cerebral microvascular oxygen partial pressure and improved cerebral oxygen delivery, Weiskopf et al [16] found that hyperoxygenation inversed anemia-induced deficits of cognitive function and memory in awake volunteers having undergone extreme hemodilution and Meier et al. [17] suggest due to their study that hyperoxygenation sustains myocardial function.

However, for our study we conclude that at normal Hct values and during moderate hemodilution hyperoxia-induced vasoconstriction impairs microvascular oxygen transport and tissue oxygenation in the peripheral microcirculation.

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Figure 1.

Figure 2.

Table 1

Oxygen delivery and consumption in the microcirculation

	43% Hct, FiO2 0.21	43% Hct, FiO2 1.00	25% Hct, FiO2 0.21	25% Hct, FiO2 1.00
Microvascular arterial flow, nL/s	1	1.07	1	0.6
Micrvascular venular flow, nL/s	1	1.6	1	0.43
Arteriolar PO ₂ , mmHg	36.4±3.8	45.0±7.6	41.3±8.5	51.2±14.2
Arteriolar saturation,%	0.59	0.73	0.68	0.8
Venular PO ₂ , mmHg	27.3±4.9	35.5±10.7	26.3±9.8	23.1±8.1
Venular saturation, %	0.39	0.57	0.36	0.28
Oxygen delivery, ml O ₂ /dl min	19.47	32.41	13.11	28.05
Relative oxygen consumption,mlO ₂ /dl	94.25	90.1	127.29	118.17

Table 2

Systemic and blood gas parameters

Parameters	43% Hct, FiO2 0.21	43% Hct, FiO2 1.00	25% Hct, FiO2 0.21	25% Hct, FiO2 1.00
MAP, mmHg	122.1±8.2	127.1±6.3	115±7.1	109.5±11.9
HR, bpm	447± 39	436±43	401±61	424±63
CO, ml/min	17.9±3.3	12.7±2.7	24.3±4.3	16.9
Cl,ml/(min*kg)	203.4±16.2	144±31	277.3±39.5	192.8
Hct, %	42.8±3.0	42±3.4	27.2±1.8	30.8±4.6
Hb,g/dl	14.6±0.8	14.6±0.8	9.4±0.5	10.4±1.5
pH	7.393±0.02	7.354±0.02	7.340±0.04	7.523±0.02
PCO2, mmHg	51.4±3.9	56.96±5.4	60.36±5.8	60.3±3.4
PO2, mmHg	66.5±7.1	412.5±26.8	64.5±7.9	451.8±10.2
BE, mmol/l	4.36±0.7	3.8±1.1	4.1±1.8	3±2.3
FCD	90±19	91±26	88±31	83±16