Effect of inhalation of different mixtures of $O_2$ and $CO_2$ on retinal blood flow

A Luksch, G Garhöfer, A Imhof, K Polak, E Polska, G T Dorner, S Anzenhofer, M Wolzt, L Schmetterer

Aim: To determine the effects of various mixtures of $O_2$ and $CO_2$ on retinal blood flow in healthy subjects.

Methods: A randomised, double masked, four way crossover trial was carried out in 12 healthy male non-smoking subjects. Gas mixtures (100% $O_2$, 97.5% $O_2$ + 2.5% $CO_2$, 95% $O_2$ + 5% $CO_2$, and 92% $O_2$ + 8% $CO_2$) were administered for 10 minutes each. Two non-invasive methods were used: laser Doppler velocimetry (LDV) for measurement of retinal blood velocity and fundus imaging with the Zeiss retinal vessel analyser (RVA) for the assessment of retinal vessel diameters. Arterial pH, pCO2, and pO2 were determined with an automatic blood gas analysis system. Retinal blood flow through a major temporal vein was calculated.

Results: Retinal blood velocity, retinal vessel diameter, and retinal blood flow decreased during all breathing periods ($p < 0.001$ each). Administration of 92% $O_2$ + 8% $CO_2$ significantly increased SBP, MAP, and PR ($p < 0.001$ each, versus baseline), whereas the other gas mixtures had little effect on systemic haemodynamics. Addition of 2.5%, 5%, and 8% $CO_2$ to oxygen caused a marked decrease in pH and an increase in pCO2 ($p < 0.001$ versus pure oxygen).

Conclusions: Breathing of pure oxygen and oxygen in combination with carbon dioxide significantly decreases retinal blood flow. Based on these data the authors speculate that hyperoxia induced vasoconstriction is not due to changes in intravascular pH and cannot be counteracted by an intravenous increase in pCO2.

Elevated arterial blood oxygen tension ($pO_2$) results in vasoconstriction and a pronounced decrease in retinal blood flow.1-3 The mechanisms underlying $O_2$ induced vasoconstriction are not completely clear. We have previously shown that endothelin receptor mediated vasoconstriction plays a part in hyperoxia induced vasoconstriction in healthy subjects.4 This is in keeping with several animal experiments,5-7 in which thromboxane A2, 20-HETE, and cytochrome P-450 were identified as other mediators of hyperoxia induced vasoconstriction. Another factor which may contribute to the reduction in retinal blood flow during hyperoxia is intracellular and extracellular alkalosis. In vitro experiments indicate that the contractile tone of pericytes strongly depends on the pH.8-10 Pericytes are, however, assumed to play a part in the regulation of vascular resistance in the retina. Hence, one may speculate that pericytes participate in the regulation of retinal capillary blood flow in response to changes in the pH.

In the present study we examined the effects of various mixtures of $O_2$ and $CO_2$ (hyperoxia-hypercapnia) on retinal blood flow in healthy subjects. Previous studies investigating this topic were contradictory.11-14 This may be related to the fact that none of these trials was masked or randomised. Moreover, in none of these clinical studies in healthy subjects was retinal perfusion assessed continuously during the inhalation periods and time dependent effects may therefore have been missed.

METHODS
Subjects
The study was performed in adherence to the guidelines of the Declaration of Helsinki and the good clinical practice (GCP) guidelines. After approval of the study protocol by the ethics committee of the Vienna University School of Medicine and after written informed consent was obtained, 12 healthy male non-smoking subjects (sample size calculation according to Stolley and Strom15: a study in 12 volunteers has the statistical power of 80% ($\beta - 1$) to detect a 2% difference in vessel diameter), were included (age 21–35 years, mean 29.0 (SD 4.8). The participants were selected by the department of clinical pharmacology. All subjects passed a prestudy screening during the 4 weeks before the first study day, which included a physical examination and medical history, 12 lead electrocardiogram, urine analysis, haematological status (haemoglobin, haematocrit, APTT, thrombin time) and an ophthalmic examination. Subjects with normal findings in the screening examinations and ametropia of less than 3 dioptres were included in the trial. In all subjects the right eye was studied.

Study design
Subjects were asked to refrain from alcohol and caffeine for at least 12 hours before trial days and were studied after an overnight fast. The study was performed in a randomised, double masked, four way crossover design. The following gas mixtures were administered for 10 minutes each: 100% $O_2$, 97.5% $O_2$ + 2.5% $CO_2$, 95% $O_2$ + 5% $CO_2$ (known as carbon), and 92% $O_2$ + 8% $CO_2$ (AGA, Vienna, Austria, certified gases for human use). All gases were delivered, after preparation by an unblinded study nurse, through a partially expanded reservoir bag at atmospheric pressure using a two valve system to prevent rebreathing. The odourless gas mixture was not identifiable for volunteers or assessing physicians.

Description of study days
Given that two different instruments were applied for measuring retinal vessel diameter and retinal blood velocity, the study had to be performed on two separate study days for technical reasons. Both study days followed an identical protocol. Four inhalation periods with different mixtures of oxygen and carbon dioxide were scheduled. Subjects were randomised at
study entry according to block randomisation with respect to inhalation of four different mixtures of oxygen and carbon dioxide and with respect to the two different study days for either measurements with the Zeiss retinal vessel analyser (RVA) or the laser Doppler velocimetry (LDV). A period of at least one day was scheduled between the two study days.

All subjects were studied with pupil dilated after instillation with tropicamide (Mydriaticum “Agepha”). An initial 20 minute resting period ensured stable haemodynamic conditions. Then systemic and retinal haemodynamic baseline values were measured. After completion an inhalation period of an O2/CO2 gas mixture was started for 10 minutes and retinal haemodynamic measurements were performed continuously throughout the inhalation period. Blood pressure and pulse rate were measured every 2 minutes. The minimum time span between two inhalation periods was 30 minutes to re-establish baseline conditions. After this resting period new baseline values were measured and the next O2/CO2 gas mixture was administered according to the randomisation list. Before and at the end of each breathing period capillary blood gas values were determined from the arterialised carotid (Falgon ointment, Thomae, Biberach, Germany) to assess pH, pCO2, and pO2.

### Methods

**Systemic haemodynamics**

Systolic, diastolic, and mean blood pressures (SBP, DBP, MAP) were measured on the upper arm by an automated oscillometric device. Pulse rate (PR) was automatically recorded from a finger pulse oximetric device (HP-CMS patient monitor, Hewlett Packard, Palo Alto, CA, USA).

**Blood gas analysis**

Arterialised capillary blood from the carotid was collected from a lancet incision into a thin glass capillary tube. Arterial pH, pCO2, and pO2 were determined with an automatic blood gas analysis system (AVL 995-Hb, Graz, Austria).

**Bidirectional laser Doppler velocimetry** (LDV)

The principle of red blood cell velocity measurement with LDV is based on the optical Doppler effect. Laser light, which is scattered by moving erythrocytes is shifted in frequency. This frequency shift is proportional to the blood flow velocity in the retinal vessel. The maximum Doppler shift corresponds to the centre line erythrocyte velocity ($V_{\text{max}}$). Using bidirectional laser Doppler velocimetry the absolute velocity in the retinal vessels can be obtained.

In the present study we used a fundus camera based system with a single mode laser diode at a centre wavelength of 670 nm (Oculix Sarl, Arbaz, Switzerland). The Doppler shift power spectra were recorded simultaneously for two directions of the scattered light. The scattered light was detected in the image plane of the fundus camera. This scattering plane can be rotated and adjusted in alignment with the direction of $V_{\text{max}}$, which enables absolute velocity measurements. LDV provides a reliable and reproducible technique for retinal blood velocity measurement.

In the present study $V_{\text{max}}$ was determined in a main inferior or superior temporal retinal vein. All measurement locations were within one to two disc diameters from the centre of the optic disc. Before data were analysed, spikes due to micro movements and blinks were removed.

#### Diameter measurement using Zeiss retinal vessel analyser (RVA)

The vessel diameters at the same measurement locations (D) were determined from retinal images recorded with a fundus camera based system. In this study the anatomical marker was either the main superior or the inferior temporal vein, in both measurements (RVA and LDV) the same measurement point was selected. The Zeiss RVA is a commercially available system which comprises a fundus camera (Zeiss FF 450, Jena, Germany), a video camera, a real time monitor, and a personal computer with an analysing software for the accurate determination of retinal arterial and venous diameters. Every second a maximum of 25 readings of vessel diameter can be obtained. For this purpose the fundus is imaged onto the charge coupled device chip of the video camera. The consecutive fundus images are digitised using a frame grabber. In addition, the fundus image can be inspected on the real time monitor and, if necessary, stored on a video recorder. Evaluation of the retinal vessel diameters can either be done online or offline from the recorded video tapes.

The system is therefore able to correct automatically for alterations in luminance as induced for instance by small eye movements. If the requirements for the assessment of retinal vessel diameters are not fulfilled anymore, as it occurs during blinks, the system automatically stops the measurement of vessel diameter. As soon as an adequate fundus image is achieved again, measurement of vessel diameters restarts automatically. Our previous data revealed excellent reproducibility of measurements with the Zeiss retinal vessel analyser.

#### Calculation of retinal blood flow ($Q$)

Blood flow through an individual major retinal vein was calculated from the results of the measurements of blood flow

### Table 1 Effect of inhalation of different mixtures of O2 and CO2 on blood pressure and pulse rate (n=12; means (SD) on both study days

<table>
<thead>
<tr>
<th></th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>PR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End of breathing period</td>
<td>Baseline</td>
<td>End of breathing period</td>
</tr>
<tr>
<td>RVA, study day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% O2</td>
<td>116 (9)</td>
<td>116 (12)</td>
<td>64 (9)</td>
<td>64 (9)</td>
</tr>
<tr>
<td>97.5% O2 + 2.5% CO2</td>
<td>121 (12)</td>
<td>121 (10)</td>
<td>68 (13)</td>
<td>65 (9)</td>
</tr>
<tr>
<td>95% O2 + 5% CO2</td>
<td>118 (12)</td>
<td>121 (11)</td>
<td>65 (9)</td>
<td>63 (11)</td>
</tr>
<tr>
<td>92% O2 + 8% CO2</td>
<td>119 (12)</td>
<td>133 (9)*</td>
<td>68 (9)</td>
<td>67 (17)</td>
</tr>
<tr>
<td>LDV, study day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% O2</td>
<td>116 (13)</td>
<td>116 (10)</td>
<td>65 (12)</td>
<td>64 (10)</td>
</tr>
<tr>
<td>97.5% O2 + 2.5% CO2</td>
<td>114 (12)</td>
<td>120 (10)</td>
<td>67 (12)</td>
<td>69 (12)</td>
</tr>
<tr>
<td>95% O2 + 5% CO2</td>
<td>115 (9)</td>
<td>122 (8)</td>
<td>67 (11)</td>
<td>68 (6)</td>
</tr>
<tr>
<td>92% O2 + 8% CO2</td>
<td>120 (12)</td>
<td>131 (13)*</td>
<td>68 (11)</td>
<td>74 (8)</td>
</tr>
</tbody>
</table>

* p<0.05 versus 100% O2.
velocity and retinal vessel diameter. The mean retinal blood velocity ($V_{max}$), in the measured vein was calculated as:

$$V_{mean} = V_{max}/1.6$$

Blood flow through this retinal vein was calculated as:

$$Q = V_{mean} \times \pi \times D^2/4.$$  

**Data analysis**

From continuous retinal vessel diameter and retinal blood velocity measurements the mean over 2 minutes was calculated for these parameters. Accordingly, six values of retinal blood velocity or vessel diameter were obtained from each breathing period, one at baseline and five during the 10 minute inhalation period.

Baseline values of all outcome variables were compared by one way ANOVA. The effect of breathing different gas mixtures on retinal haemodynamic parameters was calculated as percentage change from baseline values. Percentage changes as presented in the results section represent the maximum deviation from baseline. Relative data of retinal vessel diameter, retinal blood velocity, and retinal blood flow were used for statistical analysis. The effects of breathing different gas mixtures were assessed with four way ANOVA for repeated measures. Post hoc analysis was performed by comparing the effect of the different O$_2$/CO$_2$ mixtures versus 100% O$_2$ by using two way ANOVA for repeated measures. In order to calculate the effects of the individual doses of O$_2$/CO$_2$ versus baseline a one way ANOVA was performed. For blood pressure, pulse rate, and blood gas parameters the statistical analyses were performed using the absolute values. Data are presented as means (SD). A p value of < 0.05 was considered the level of significance.

**RESULTS**

Two subjects reported transient headache after breathing 92% O$_2$ + 8% CO$_2$, no other adverse events were observed during the study. In all subjects blood pressure and pulse rate returned to baseline levels immediately after cessation of the breathing periods.

Baseline values and systemic effects of different mixtures of O$_2$ and CO$_2$ breathing on blood pressure and pulse rate are shown in Table 1. There were no significant differences between the baseline values of systemic haemodynamics on the two trial days. Administration of 92% O$_2$ + 8% CO$_2$ significantly increased SBP (+15% (9%)) during RVA measurements and 14% (8%), during LDV measurements p < 0.001 each), MAP (RVA: +11% (8%); LDV: +11% (13%), p < 0.001 each) and PR (RVA: +19% (11%); LDV: +13% (19%), p < 0.001 each versus baseline) on both trial days. This effect of 92% O$_2$ + 8% CO$_2$ was also significant versus 100% O$_2$ breathing alone for SBP (p < 0.001), MAP (p = 0.009), and PR (p < 0.001), but not for DBP. The other gas mixtures had no consistent effects on blood pressure or PR.

Baseline values of all blood gas parameters were comparable on both study days. The same holds true for the effects of pH, pCO$_2$, and pO$_2$, which are shown in Table 2. As expected, a pronounced increase in pO$_2$ (p < 0.001 versus baseline) was observed during all breathing periods. However, this response to hypoxia was comparable during all inhalation periods.

The effect of inhaling different gas mixtures on pH and pCO$_2$ was dose dependent (Table 2). The addition of 2.5%, 5%, and 8% CO$_2$ caused a marked decrease in pH (p < 0.001 each) and an increase in pCO$_2$ (p < 0.001 versus baseline) was observed during all breathing periods. However, this response to hyperoxia was comparable during all inhalation periods.

As demonstrated in Table 3, baseline values of RVA or LDV were similar before each breathing period. Figure 1 shows the time course of retinal venous diameter, retinal blood velocity, and retinal blood flow during the breathing periods. Inhalation of all gas mixtures induced a decrease of retinal vessel

| Table 2 Effect of inhalation of different mixtures of O$_2$ and CO$_2$ on systemic pH, pCO$_2$, and pO$_2$ (n=12; means (SD)) on both study days |
|-----------------------------|-----------------------------|-----------------------------|
|                            | RVA, study day               | LDV, study day               |
|                            | Baseline                     | End of breathing period      | Baseline                     | End of breathing period      |
| pH                          |                             |                             |                             |                             |
| 100% O$_2$                  | 7.38 (0.02)                 | 7.39 (0.03)                 | 7.35 (0.08)                 | 7.37 (0.09)†††               |
| 97.5% O$_2$ + 2.5% CO$_2$   | 7.38 (0.03)                 | 7.37 (0.03)                 | 7.34 (0.09)                 | 7.33 (0.09)†††               |
| 95% O$_2$ + 5% CO$_2$       | 7.38 (0.02)                 | 7.34 (0.02)                 | 7.36 (0.07)                 | 7.32 (0.08)†††               |
| 92% O$_2$ + 8% CO$_2$       | 7.38 (0.02)                 | 7.27 (0.04)                 | 7.34 (0.07)                 | 7.23 (0.09)†††               |
| pH                          | 4.09 (0.00)                 | 4.10 (0.00)                 | 4.07 (0.00)                 | 4.09 (0.00)                 |
| pCO$_2$                     | 5.08 (0.21)                 | 5.10 (0.21)                 | 5.07 (0.21)                 | 5.09 (0.21)                 |
| pO$_2$                      | 85 (7)                      | 85 (7)                      | 85 (7)                      | 85 (7)                      |
| pCO$_2$                     | 40 (2)                      | 40 (2)                      | 40 (2)                      | 40 (2)                      |
| pO$_2$                      | 9.7 (4.0)                   | 9.7 (4.0)                   | 9.7 (4.0)                   | 9.7 (4.0)                   |

*Denotes RBF through one specific vein and not total RBF.

| Table 3 Baseline values of retinal blood flow (RBF), vessel diameter (D), and retinal blood velocity (Vel) (n=12; means (SD)) before inhalation of different mixtures of O$_2$ and CO$_2$ |
|-----------------------------------------------|-------------------------------|-------------------------------|
| RBF* (µl/min)                               | D (µm)                        | Vel (cm/s)                    |
| Baseline                                    | Baseline                      | Baseline                      |
| 100% O$_2$                                  | 2.4 (1.7)                     | 1.5 (1.7)                     |
| 97.5% O$_2$ + 2.5% CO$_2$                   | 2.5 (1.8)                     | 1.5 (1.8)                     |
| 95% O$_2$ + 5% CO$_2$                       | 2.6 (1.9)                     | 1.5 (1.9)                     |
| 92% O$_2$ + 8% CO$_2$                       | 2.7 (2.0)                     | 1.5 (2.0)                     |
ishes the oxygen induced vasoconstriction in the retina. The flow during combined O2 and CO2 breathing. Our results concludes that this is the result of the time course of retinal blood flow. Consequently neither 5% nor 8% CO2 abolishes retinal vessel diameter, retinal blood velocity, and retinal blood flow versus baseline (p <0.001 each). This decrease was indicated by carbonic anhydrase. In the same experiments it was also implied retinal vasoconstriction based on the observation that 8% CO2 + 92% O2 reduced resistance index as assessed with colour Doppler imaging, but we have recently shown that this index is not an adequate measure of vascular resistance in the retina. Finally, an increase in retinal blood flow during carbogen breathing has been proposed from measurements using the blue field entoptic technique. One has to be careful, however, to draw any conclusions on retinal blood flow from this study, because leucocyte rather than erythrocyte movement is assessed with this technique. Our results are, however, compatible with the idea that hypercapnia without hyperoxia induces vasoconstriction as evidenced from a variety of studies.

Interpreting our results during 8% CO2 + 92% O2 breathing one has to consider the increase in blood pressure during this stimulus. Whereas the retina is auto-regulated over a wide range of perfusion pressures, hypercapnia may narrow the autoregulatory plateau as it does in the brain. Hence, the increase in ocular perfusion pressure during 8% CO2 + 92% O2 may well contribute to the response in retinal blood flow during this stimulus.

A technical limitation of the present study is that red blood cell velocity cannot be recorded simultaneously with vessel diameter because these parameters are evaluated by two different methods. Hence, we have measured retinal vessel diameter and retinal blood velocity on two different study days. At both study days the same subjects were studied and baseline conditions were comparable. In addition, comparable effects of the gas mixtures on systemic haemodynamics were achieved. To obtain adequate results with the methods employed, it is necessary that the subjects under study are concentrated and collaborate with the instructions of the investigator. As breathing higher carbon dioxide concentrations is exhausting, it was an advantage to schedule two study days in the present study.

The present study may be of interest with respect to the mechanism underlying hyperoxia induced vasoconstriction and indicates that alkalois does not have a major role. On the one hand the effects of a more than fourfold elevation in pO2 levels on pH were small, whereas pronounced vasoconstriction in the retina was achieved. On the other hand adding CO2 to the inhalate induces acidosis but does not abolish hyperoxia induced retinal blood flow changes. In other words the degree of vasoconstriction was not coupled with changes in pH in the present study. This indicates that during systemic hyperoxia potent vasoconstrictors are produced in the retina, which clearly dominate the regulatory potential of acidosis.

These results, however, do not preclude that extravascular acidosis in the retina may counteract hyperoxia induced vasoconstriction. In the present study we measured blood gas values from capillary blood samples of the arterialised earlobe and there is no reason to assume that pO2, PCO2 and pH differ largely between arteries in the ear and in the eye. There is, however, evidence that the pH of retinal tissue may considerably differ from intravascular pH in retina arteries. During normocapnia retinal pH in the pig is more acidic (7.22) than arterial pH (7.44) as evidenced by studies using microelectrodes. CO2 was considered the main factor affecting retinal pH in these experiments, because it easily diffused through retinal vessels to the locations where it is hydrolysed by carbonic anhydrase. In the same experiments it was also observed that the first part of the response slope to CO2 breathing was steep and linear (after 3–4 minutes) reaching a plateau after 8–10 minutes. This indicates that the selected
duration of the breathing periods in our study was adequate to reach steady state conditions. Hence, the present data indicate that intravascular pH is not an important factor in mediating oxygen induced vasconstriction in the human retina. Whether this is also true for pH in the inner retina can not be answered from the present study, because of the obvious methodological limitations of human trials.

In conclusion, the present study shows that breathing of pure oxygen and oxygen in combination with carbon dioxide significantly decreases retinal blood flow. Based on our data we speculate that hyperoxia induced vasconstriction is not due to changes in intravascular pH and cannot be counteracted by an intravascular increase in pCO₂.

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