Aqueous oxygen: the solution to relief hypoxic pulmonary hypertension

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Abstract

Objective: To evaluate the effects of hyperbaric oxygen solution on hypoxic pulmonary hypertension. Methods: Eleven calves, 2-month-old, 71 ± 6 kg, underwent general anaesthesia, mechanical ventilation and median sternotomy. Catheters for continuous pressure and blood gas measurements were inserted in carotid and femoral arteries, left atrium, right atrium and pulmonary artery (PA), and a flow-probe placed around the PA. After baseline measurements 30 min hypoxic ventilation reduced the mean arterial PO2 from 285 ± 115 to 46 ± 11 mmHg (P < 0.0001). At this point, without changes in hypoxic ventilation (mean arterial PO2 maintained at 50 ± 5 mmHg), 3 ml/min of hyperbaric aqueous oxygen (AO, oxygen diluted in saline solution) was infused directly into the PA for 30 min, with continuous reading of the monitored parameters. Results: Hypoxic ventilation raised significantly (P < 0.005) the values of systolic (36 ± 7 vs 22 ± 6 mmHg), diastolic (16 ± 3 vs 9 ± 4 mmHg) and mean (24 ± 4 vs 14 ± 4 mmHg) PA pressure, PA/systemic pressure ratio for systolic (0.47 ± 0.09 vs 0.24 ± 0.06) and mean (0.49 ± 0.13 vs 0.23 ± 0.08) pressures and Pulmonary Vascular Resistance (PVR) (6.89 ± 0.87 vs 2.67 ± 0.38 U), while the Pulmonary Blood Flow (PBF) decreased (2.7 ± 0.4 vs 3.7 ± 0.4 l/min). AO infusion reduced significantly (P < 0.005) the values obtained with hypoxic ventilation with systolic (26 ± 6 vs 36 ± 7 mmHg), diastolic (11 ± 4 vs 16 ± 3 mmHg) and mean (16 ± 4 vs 24 ± 4 mmHg) PA pressure, PA/systemic pressure ratio for systolic (0.27 ± 0.07 vs 0.47 ± 0.09) and mean (0.27 ± 0.08 vs 0.49 ± 0.13) pressures and PVR (3.42 ± 0.31 vs 6.89 ± 0.87 U), while the PBF increased (3.6 ± 0.4 vs 2.7 ± 0.4 l/min). Conclusions: Acute infusion of hyperbaric AO solution into the PA completely reverses the negative effects of acute hypoxia on pulmonary circulation. © 2004 Elsevier B.V. All rights reserved.

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1. Introduction

Pulmonary hypertension can be primary, when no evident cause can be identified, or secondary to a well defined lesion: congenital heart defect with left-to-right shunt and increased pulmonary blood flow, pulmonary venous hypertension, disorders of the respiratory system, hypoxemia, chronic thrombotic and/or embolic disease, and a variety of miscellaneous causes [1,2].

While the pulmonary arterial vessels constrict in response to acute hypoxia, prolonged exposure to low oxygen environment leads to structural remodelling of these vessels, including thickness of adventitial and medial layers and muscularization of pre-capillary vessels [3]. The combination of pulmonary vasoconstriction and vascular remodelling, coupled with increased hematocrit, results in pulmonary hypertension and subsequently right ventricular hypertrophy [4,5].

In patients with congenital heart defects the severity of the vascular remodelling in the pulmonary vessels appears in direct correlation with the increase of the pulmonary blood flow, the pressure and oxygen saturation at whom it is delivered, and the duration [6].

Prolonged pulmonary hypertension may result in irreversible vascular lesions, leading to pulmonary vascular obstructive disease and the corresponding clinical pattern of the Eisenmenger syndrome [7–10].

It is well recognized that the currently available treatments for pulmonary hypertension, including continuous oxygen administration and vasodilator therapy with either Calcium antagonists, Prostaglandins and Nitric Oxide, are unsatisfactory, with the exception of specific acute or chronic circumstances [1].

This experimental study has been designed to test the hypothesis that hypoxic pulmonary hypertension can be reduced by delivering hyperbaric oxygen solution directly into the pulmonary circulation.

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2. Materials and methods

After induction of general anesthesia eleven calves, 2 month old, 71 ± 6 kg, underwent tracheal intubation, mechanical ventilation and chest opening through median sternotomy.

Catheters for continuous pressure and blood gas measurements were directly inserted in carotid and femoral arteries, left atrium, right atrium and pulmonary artery, and a flow-probe was placed around the main pulmonary artery (Fig. 1). The presence of intra-cardiac shunts as well as of patent ductus arteriosus was ruled out at the beginning of the experiments.

2.1. Instrumentation

- **Pressures measurement:** Continuous pressure values were obtained connecting the systemic and pulmonary artery catheters, as well as the right and left atrial catheters, to high fidelity pressure probes (Millar Mikro-Tip, model MPC-500) with a pressure range of −50 to 300 mmHg and a sensitivity of 5 μV/V/mmHg. The ratio between pulmonary artery and systemic pressure for values of systolic and mean pressures was calculated.

- **Flow-meter measurements:** T206 blood flow-meter probes (Transonic Systems Inc., Ithaca, NY) were used, 12–16 mm and 20–27 mm in size (accordingly with the size of the pulmonary artery), with flow accuracy of 1%, resolution of 1 ml/min and flow sample rates 333 Hz.

- **Blood gases measurements:** pH, oxygen and carbon partial pressure from the right and left atrial catheters were continuously measured and displayed with an in-line gas monitoring system (CDL-500, Terumo, 3M Health Care, Ann Arbor, MI) and the data read and recorded with a data acquisition system (LabView 6, National Instruments, Austin, TX). Blood gases were double checked in correspondence of every recording of the hemodynamic parameters with analysis (ABL 700 analyzer, Radiometer, Copenhagen, Denmark) of blood samples obtained from right atrium and carotid artery, respectively for the pulmonary and systemic circulation.

- **Pulmonary vascular resistance:** The value was calculated with the following formula:

  
Pulmonary vascular resistance = (mean pulmonary artery pressure − mean left atrial pressure)/pulmonary blood flow.

After the baseline measurements, hypoxic pulmonary hypertension was obtained through 30 min of hypoxic ventilation, reducing the mean arterial PO2 from 285 ± 115 to 46 ± 11 mmHg (P < 0.0001).

At this point, without any change in the hypoxic ventilation (the mean arterial PO2 was maintained at 50 ± 5 mmHg, NS), 3 ml/min of hyperbaric aqueous oxygen (AO, oxygen diluted in saline solution with a concentration of 0.82 ml of oxygen/ml Lactated Ringer’s solution, TherOx Inc., Irvine, California) were added to 72 ml/min of blood withdrawn from the carotid artery and, via a custom syringe pump (TherOx Inc., Irvine, California), infused directly into the pulmonary artery (Fig. 1) for 30 min, with continuous reading of the monitored parameters.

At the end of the 30 min with AO infusion (Fig. 2), after new samples for blood gases were taken and all
the parameters were recorded, the experiments were ended and the animals sacrificed.

All animals received human care in compliance with the ‘Principles of Laboratory Animals’ formulated by the National Society of Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication 85-23, revised 1985). The protocol was approved by the institutional Committee on Animal Research.

Student’s t-test has been used for statistical evaluation. Data are expressed as mean ± SD. The significance level was P = 0.05.

3. Results

In all experiments 30 min of hypoxic ventilation raised significantly the values of systolic, diastolic and mean pulmonary artery pressure, pulmonary artery/systemic pressure ratio for systolic and mean pressures and pulmonary vascular resistance, while the pulmonary blood flow decreased (Table 1).

After other 30 min of the same hypoxic ventilation, but with AO infusion, all the values obtained with hypoxic ventilation only (systolic, diastolic and mean pulmonary artery pressure, pulmonary artery/systemic pressure ratio for systolic and mean pressures and pulmonary vascular resistance) returned to be not statistically different from baseline values obtained without hypoxia (Table 1).

The pulmonary vascular resistance with AO decreased to a value lower than with hypoxic ventilation only (3.42 ± 0.31 vs 6.89 ± 0.87 U/m², P < 0.0001), but remained higher than the value obtained with normal ventilation (2.67 ± 0.38 U/m², P < 0.01) (Table 1).

4. Discussion

Oxygen administration by ventilation either may fail to correct arterial hypoxemia with the associated pulmonary hypertension or may be limited by the potential for pulmonary toxicity at high inspired oxygen concentration [11].

The only possibility currently available for introducing oxygen into blood requires its diffusion across an artificial gas–liquid interface. Mass transport of oxygen by diffusion is inherently slow, so that a relatively large surface area for contact of the two phases is required in both the extracorporeal and intravascular oxygenators [12–14]. Therefore such devices are inherently bulky, and prolonged contact with blood over a broad surface area may be associated with a variety of complications [15–16].

A bubbleless method has been developed for introducing oxygen, dissolved at elevated partial pressures in physiological crystalloid solutions (Aqueous Oxygen, TherOx Inc., Irvine, California), at rapid velocity through capillary tubes in vitro into host liquids at ambient pressure [17].

Aqueous Oxygen is a recently discovered liquid phase combination of water and medical grade oxygen that can be mixed with blood at ambient pressure to correct hypoxemia or produce hyperoxemia with a small amount of carrier solution [18].

Intravascular administration of hyperbaric oxygen during myocardial reperfusion has been shown to reduce the myocardial injury associated with ischemia/reperfusion in both experimental [19–21] and clinical studies [22–23]. Recently intravascular administration of hyperbaric oxygen has been successfully proved also as a chemotherapy adjuvant in the treatment of metastatic lung tumors in a rat model [24].

In this experimental study the administration of hyperbaric oxygen solution directly into the pulmonary circulation, despite the minimal amount utilized (3 ml/min mixed with 2.7 ± 0.4 l/min of pulmonary blood flow measured during the hypoxic ventilation), allowed for the correction of all the hemodynamic parameters negatively affected by the hypoxic ventilation, with return to the values obtained at baseline with normal ventilation.

Our experimental observations are consistent with our hypothesis that a minimal amount of oxygen is sufficient to reverse the pulmonary vasoconstriction induced by severe

| Table 1 |

<table>
<thead>
<tr>
<th>Hemodynamic parameters</th>
<th>PA pressure (mmHg)</th>
<th>PA/systemic ratio</th>
<th>PBF (l/min)</th>
<th>PVR (U/m²)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
<td>Mean</td>
<td>Systolic</td>
</tr>
<tr>
<td>Baseline</td>
<td>22 ± 6</td>
<td>9 ± 4</td>
<td>14 ± 4</td>
<td>0.24 ± 0.06</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>36 ± 7</td>
<td>16 ± 3</td>
<td>24 ± 4</td>
<td>0.47 ± 0.09</td>
</tr>
<tr>
<td>AO</td>
<td>26 ± 6</td>
<td>11 ± 4</td>
<td>16 ± 4</td>
<td>0.27 ± 0.08</td>
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<tr>
<td>Baseline vs hypoxia</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0005</td>
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<tr>
<td>Hypoxia vs AO</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.0005</td>
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<tr>
<td>Baseline vs AO</td>
<td>NS</td>
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AO, aqueous oxygen; PA, pulmonary artery; PBF, pulmonary blood flow; PVR, pulmonary vascular resistance.
hypoxia, provided that the oxygen is administered directly into the pulmonary circulation.

Of course we are aware that the amount of 3 ml/min used in our study could represent a substantial volume overload where applied to a subject with a much smaller body weight and size. In view of potential clinical applications in a subject like a neonate or a small infant, one or more of the currently available solutions to reduce the volume overload should be taken into consideration.

4.1. Study limitations

(a) Only the acute effects of AO administration have been studied, in an acute situation with pulmonary hypertension artificially created with 30 min of hypoxic ventilation. The consequences of AO administration after induction of chronic hypoxia [4] or in the presence of established pulmonary hypertension with histological pulmonary vascular lesions [6] should be evaluated.

(b) Despite the evident experimental benefits of AO administration on the hemodynamic parameters, reactive oxygen species generation upon the intravascular introduction of oxygen remains a potential concern. Despite the relationship between oxygen partial pressure in the reperfused tissues and reactive oxygen species production is likely to be quite complex, nevertheless it is possible that oxygen toxicity may occur with longer periods of treatment.

4.2. Conclusions

Our experimental study demonstrated that acute infusion of hyperbaric AO solution into the pulmonary circulation can completely reverse the negative hemodynamic effects of acute hypoxia. These observations can lead to the extension of the experimental studies with the AO utilization in more severe and chronic situations, in order to evaluate the potential clinical applications of this new available technology. ‘If the results observed with acute hypoxia will be confirmed in a chronic hypoxic model, the potential clinical application of administering the hyperoxic solution into the pulmonary circulation through a simple central venous line could be taken into consideration’.

References


Appendix A. Conference discussion

Dr A. Wechsler (Philadelphia, PA, USA): I didn’t notice in the abstract, What was the effect of infusing the aqueous hyperbaric oxygen on left atrial oxygen saturation?

Dr Corno: We did not report it in the abstract, nor in the presentation, but there were no changes in the left atrial pressure. The mean left atrial pressure didn’t change significantly. There was only a minimal increase during the period of hypoxia, but not significant.

Dr Wechsler: And oxygen saturation in the left atrium, it went way up?

Dr Corno: The saturation in the left atrium was exactly the same saturation as in the aorta. It was due to the hypoxic ventilation. So it was definitely at the same level. We never detected any difference between left atrium and femoral artery where we were drawing the blood sample, because there were no intracardiac shunts. We had to exclude intracardiac shunt or patent ductus arteriosus before the study, obviously. So the left atrial saturation was always the same as the aortic saturation.

Dr Wechsler: It seems like an interesting opportunity to assess what would have been the effects of increasing the oxygen saturation in the left atrium rather than the pulmonary artery and seeing if there were changes in pulmonary artery pressure by that maneuver, in an attempt to define the receptors responsible.

Dr Corno: Well, you mean returning the animal to the same baseline, 100 percent of the saturation?

Dr Wechsler: Yes.

Dr Corno: Well, we have done this and everything returned to the baseline. Since the cardiac hypertension was due to pulmonary vasoconstriction subsequent to hypoxia, simply reversing the hypoxic ventilation to normoxic ventilation, we returned to the baseline values.

Dr Wechsler: And this was an aqueous carrier. Had you done similar experiments with a Fluosol or some agent such as that?

Dr Corno: No. We wanted to keep it with normal saline exactly because, theoretically, once we will have all the data for administration after chronic hypoxia, then we can move to the clinical application. It is an ideal treatment for any patient having pulmonary hypertension, because any patient is already having an arterial line and a central venous line, and simply with any normal saline solution, if you can add oxygen and infuse oxygen in the right atrium through the central venous line, you can reduce the pulmonary vasoconstriction.

Dr M. Turina (Zurich, Switzerland): Can you help us with the physics of this experiment. If you put hyperbaric oxygen and then you’re infusing the pulmonary artery under normal atmospheric pressure, this oxygen, which is hyperbaric, will immediately build microbubbles. You speak of diluting the oxygen with fluid. You cannot dilute gas with a fluid, it’s physically impossible. So in what form is this oxygen finally when exposed to the normal pressure in the pulmonary artery?

Dr Corno: I don’t have the complete answer how is it possible to dilute the oxygen in normal saline, because this is what happens in the cartridge, which I call the Chamber of Secrets, and it’s protected by the company.

But the solution coming out of this mixing chamber with high oxygen content is mixed with the blood of the animal in this case. That means we are drawing 72 ml/min of blood from the patient, mixed with 3 ml of this hyperoxygenated solution and reinfusing it into the pulmonary artery for 75 ml/min.

Dr Turina: But you have a situation like opening a bottle of champagne. When you reduce the pressure, it will come out in bubbles. So that’s what I don’t understand, the physics of the whole thing. Either there must be some emulgaror inside or it will come in microbubbles. Did you have an ultrasonic probe to see if there is any microbubbles in the left atrium?

Dr Corno: Evidently, they organized very well in this mixing chamber, because we didn’t detect any microbubble. And we measured the PO2 of the blood infused into the pulmonary artery, and it was between 600 and 800 mmHg.

Mr G. Gobbi (TherOx, Inc.): If I may provide some explanation for this. My name is Giorgio Gobbi. I’m the European manager for the company, TherOx, that manufactures the device.

If you can go back to the slide showing the cartridge. We are able, inside the cartridge that manufactures the oxygen solution, we inject oxygen-enriched physiologic solution through a very small capillary, which you see here. At very high pressure, we inject this physiologic solution mixed with oxygen. We have 30 bars here, of pressure of 100% oxygen, and we create the solution by spraying creating a fine mist of a physiologic solution into this 30 bar chamber.

And then the oxygenated solution is then sprayed at very high pressure through this capillary, which is a glass capillary that’s very fine inside. So unlike the glass where bubbles are produced in the champagne example you mentioned, this glass is extremely polished inside, and the internal diameter of this capillary is less than 1 micron.

So we do not create any situation for the creation of cavitations, or nucleation phenomena. So bubbles that are formed, because bubbles are still formed, but they’re nanobubbles and they are insignificant from a clinical standpoint. And so the solution ends up being very stable at ambient pressure.

Dr J. Vaage (Oslo, Norway): I would like to follow up. I have some of the same limitations as Marko Turina has.

But I’m missing a control group where you actually just have the animals hypoxic in one group and then give aqueous oxygen. And then, when you take away the aqueous oxygen and follow the animals for some time, is it possible to see any effects on the pulmonary vascular resistance? I mean, I would expect to have maybe some reactive oxygen species made here that could have a deleterious effect in the pulmonary circulation.

Dr Corno: I didn’t present the data, but we tried at the beginning to continue the protocol, as you say, by stopping the aqueous oxygen infusion, returning the animals to the normoxic ventilation, and all the parameters returned exactly as before.