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The "Normobaric Oxygen Paradox": a new tool for the anaesthetist?

Rocco M [1], D'Itri L [2], De Bels D [3,4], Corazza F[4] Balestra C [5]

[1] Intensive Care Department, La Sapienza University of Rome, Italy

[2] OTI medicale, Vicenza, Italy

[3] Intensive Care Department, Brugmann University Hospital, Brussels, Belgium

[4] Immunology laboratory, Brugmann University Hospital, Brussels, Belgium

[5] Environmental and Occupational Physiology Laboratory, Haute Ecole Paul Henri Spaak, Brussels, Belgium

Corresponding author:

Monica Rocco, M.D., Ph.D.

Associate professor of anaesthesiolgy

Faculty of Medicine and Dentistry

Università degli Studi di Roma "La Sapienza"

Rome, Italy.

e-mail: monica.rocco@uniroma1.it

Telephone number: +393356688074

Abstract

Hypoxia is the natural trigger for endogenous EPO production but recently the use of intermittent hyperoxia to stimulate EPO has been postulated and this phenomenon has been called the “normobaric oxygen paradox” (NOP).

The "NOP” is a mechanism by which oxygen regulates the expression of the Hypoxia Inducible Factor 1 alpha (HIF-1α). The HIF-1α-depending gene regulation is responsible for many different genetic expressions including EPO and VEGF.

It has been proposed that relative changes of oxygen availability rather than steady state hypoxic or hyperoxic conditions, play an important role in HIF transcriptional effects. According to this hypothesis, the cell interprets the return to normoxia after a hyperoxic event as an oxygen shortage, and induces HIF-1-regulated gene synthesis, including EPO. Being both a hormone and a cytokine, the actual actions of EPO are complex; its clinical utility has been postulated for neuroprotection and cardioprotection.

The precise level of inspired oxygen and the exact timeframe for its iterative administration are not totally known. N-Acetyl-L-Cysteine (NAC) supplementation has been shown to help.

All the reported data demonstrate how hyperoxic and hypoxic states can potentially be manipulated if oxygen is been considered as a multifaceted molecule more than just a gas.

Key words

Oxygen, hypoxia, hyperoxia, erythropoietin, normobaric oxygen paradox

Introduction

There is a great concern regarding the increased need for blood products in an aging population and the cost of safe transfusion in developing world. The use of blood red cell precursor enhancers like exogenous erythropoietin (EPO) is extensively approved not only for treating anaemia resulting from chronic kidney disease and myelodysplasia (as a side effect from the treatment of cancer by chemotherapy and radiation), but also as an integral part of the preoperative autologous blood donation program for orthopaedic surgery [1].

Renal tissue hypoxia is the only widely accepted trigger for EPO production [2-3]. Intermittent hypoxia is the natural trigger for endogenous EPO production [4] and has been widely used by athletes for many years. Recently, the use of intermittent hyperoxia to stimulate EPO has been postulated [5] and this phenomenon has been called the "normobaric oxygen paradox".

Being both a hormone and a cytokine the actual actions of EPO are complex; its clinical utility has been postulated for neuroprotection [6-7] and cardioprotection as a preoperative treatment [8] as well as in the treatment of septic patients [9].

The repetition of this simple stimulus has been used to increase haemoglobin and reticulocytes in anaemic patients due to a variety of causes opening interesting possibility in the field of alternative techniques to blood transfusion [10-12].

The normobaric oxygen paradox

The mechanism proposed to explain such phenomenon lies deep in the fundamental cellular mechanisms of adaptation to hypoxia. This latter depends on oxygen free radicals availability. In fact, in the presence of reactive oxygen species and under normoxic conditions, the hypoxia Inducible Factor 1 alpha, (HIF-1 alpha) is hydroxylated by prolyl-hydroxylase. This results in its ubiquitylation by the Von Hippel Lindau tumour suppressing Protein (VHLp) and finally in the degradation of HIF-1 alpha into the proteasome (Figure 1, panel A). In case of limited availability or absence of reactive oxygen species, the HIF-1 alpha will not link with VHLp and thus can be dimerized with HIF-1 beta. This HIF complex can bind to target promoters known as hypoxia responsive elements leading to the transcription of the erythropoietin gene as well as many other HIF-dependant genes involved in cellular metabolism [13-14].

Increasing the level of oxygen breathed by the patient (namely increasing the availability of oxygen reactive species in the cell) will enhance the production of protective agents against oxygen reactive species (ROS). This will be achieved by increasing the glutathione synthethase enzyme activity (gamma glutamyl cysteine synthethase).

This enhanced activity will thus increase the glutathione production and subsequently ROS scavenging. During the hyperoxygenation period, an increased stock of reduced glutathione (GSH) will be formed (figure 1 panel

B). After cessation of hyperoxygenation, this increased stock of GSH, together with the (slow) reduction of GSSG to GSH, produces an excess of this complex and allows the enhanced scavenging of ROS to last longer after oxygen level reduction with concomitant reduction of ROS availability [15-16] (figure 1 panel C). This scenario is responsible for the observed "normobaric oxygen paradox"[5] : a normobaric hyperoxic state causes a fall in reactive oxygen species level, sustained beyond the exposure period, triggering, at his interruption, the mechanism for EPO production. In other words the "relative" hypoxia, obtained after a period of hyperoxia, acts as a hypoxic trigger able to significantly increase EPO. Hyperbaric oxygen (HBO) breathing, on the contrary, appears to be a very effective depressor of serum EPO levels up to 24 h HBO treatment [5]. During hyperbaric oxygenation, indeed, an excessive presence of ROS and a limited increase of GSH production occurs and most of the GSH is converted to GSSG; after return to normoxia, and during the time needed for GSSG to be converted back to GSH (limiting enzyme: GSH reductase), ubiquitination of all intracellular HIF-▪ occurs, effectively blocking the transcriptional response pathway for a sustained period of time.

Method

A Medline (PubMed, U.S. National Library of Medicine of the National Institutes of Health) search was performed on January 30, 2013. Vocabulary words used were "normobaric oxygen paradox" from 1966 through to 2013. The search retrieved 13 articles. When the words "hyperoxia" AND "erythropoietin production" were used, the search retrieved 15 articles. Articles were analysed if they has a direct or indirect impact on anaesthesia. Some authors' unpublished study data were used when needed as practical examples.

Literature

Since the first hypothesis and findings of increased EPO after breath-hold diving, the explanation of hypoxia as a trigger was questioned because the diver during this kind of dive is not hypoxic to trigger EPO production.

Since the dives were performed at 40 m depth (5 atmospheres of absolute pressure), the next step was to apply the same amount of oxygen in standard conditions (laboratory conditions) to measure whether the return back from the increased oxygen partial pressure was considered by the body as a drop of oxygen in the tissues and thus leading to an increase in EPO like ascending for a stay in altitude: it was the first report presenting the possibility to increase erythropoietin with a single non hypoxic stimulus (explained in [5]).

A further study by our group has shown that normobaric oxygen, given at too high concentrations or even too often, is not as effective in increasing EPO or haemoglobin [17]. The minimal concentration of inspired oxygen as already stated, seems to lay around 40%-50%, increasing the inspired oxygen fraction to 100% indeed shows very variable and less consistent results[18].

Further investigations to determine the optimal "dose" are evidently welcomed, and the recent publications confirm the fact that giving 100% oxygen is not always optimal [19-20]. Furthermore, a too high variation of oxygen partial pressure such as achieved by moving from normobaric hyperoxia to hypoxia, seems to be likewise suppressive of EPO, as was recently published [21].

Our recent experiences on HUVEC cells (Human Umbilical Vein Endothelial Cells) show a decrease of HIF-1 alpha expression after two hours of hyperoxia reaching 0,59 % of control values, followed by 4 hours post hyperoxia by a reactive increase up to 119,1 % and 176,6 % six hours following hyperoxia as compared to basal levels [22]. No absolute hypoxia was applied to the cellular culture lines so hyperoxia was the only trigger for the increased HIF expression [23].

This increase of HIF-1 alpha allows the opportunity to propose two concomitant mechanisms for the NOP. The first favours the GSH synthethase and the other favours the increased of HIF. Of course the coexistence of both cannot be excluded (figure 2). This complex situation will allow the HIF dimers to bind and start gene expression. In our experience, this mechanism has been useful in patients with various forms of anaemia including bone marrow hypoplasia allowing a clinically significant increase of haemoglobin in these patients [10-11]. EPO has also a broad spectrum of tissue protecting actions [24]. This has been advocated in different cell types, mainly in cardiac and neural cells [6, 25].

We studied twenty-four healthy male students, divided into 2 groups (FiO_2: 15%, and 100%), breathing the oxygen blend for only 30 min every other day for a period of 10 days (5 sessions).

Haemoglobin levels and haematocrit were analysed before every session. Both temporary hypoxic and hyperoxic treatment, at least given with such a short-time protocol[22], was associated with a similar increase of Hb levels.

This again corroborates the hypothesis of the NOP which shows that the "relative" hypoxia acts as a hypoxic trigger [5, 17, 26].

Two case reports illustrate these concepts. The first case describes the application of normobaric oxygen breathing sessions over several days in a 42 yrs old woman who had developed anaemia after receiving chemotherapy for breast cancer. Daily oxygen administration was used without. Haemoglobin increased from 8 to 12 g/dL. Stopping oxygen administration decreased Hb and resuming administration re-increased it. The second case is that of a 71-year-old woman suffering from myelofibrosis undergoing an aortic valve repair. Pulsed oxygen therapy was given on top of EPO and intravenous iron. The Hb level increased in 60 days to reach 13,5 g/dl and again stopping oxygen administration decreased Hb and resuming administration re-increased it. Figure 2 shows the regression line for both patients with bone marrow impairment [26].

In orthopaedic surgery our group has just published a study in patients undergoing traumatic hip surgery.

The population was divided in two groups (room air breathing and 100% oxygen breathing.) We found a significant increase ($p<0.001$) of reticulocyte count at day 7 after as well as a reduction in the hospital length of stay in the oxygen group. There was a decrease in blood transfusion needs in the oxygen group unrelated to the NOP phenomenon, the time frame being too short [12].

In cardiac surgery [18], we have followed patients undergoing CABG or valvular replacements. They were divided into two groups upon arrival in ICU (Group 1: 50% and group 2: 100 % FiO2). Endogenous EPO concentrations tended to increase more in Group 1 than in Group 2, although the difference was not statistically significant between the two groups. However, the slope of the increase in the EPO plasma level was significantly higher in Group 1 than in Group 2 ($p= 0.0052$).

Although the Normobaric Oxygen Paradox (NOP) mechanism seems to be proven, our experience demonstrates that to effectively show a significant increase of EPO after one single exposure to normobaric oxygen is not so easy [19-27]. This matter remains controversial for a number of reasons; let's consider some of them since several concomitant mechanisms are involved here.

The individual circadian rhythm of erythropoietin production

Hormonal variations are depending on circadian as well as seasonal rhythms even though this has been questioned [28]. To show an increase at a specific moment of the day we found that a simple correction for baseline levels matching was not sufficient and an individual matching of the circadian rhythm was needed [5]. The modest acute increase of EPO was shown effective to increase haemoglobin when repeated for several days [11].

The effective amount and activity of glutathione of the subjects

In some individuals, we could not find a clear increase of EPO, usually those subjects who were older or physically inactive. This may be related to a decreased content in intracellular glutathione. It has recently been shown that repletion of glutathione intracellular reserve can be effective on EPO production. Supplementing N-Acetyl-L-Cysteine (NAC) was useful in young healthy subjects to increase EPO after 8 days of treatment, without oxygen sessions [29]. This approach has also been shown effective by a single oxygen session with concomitant supplementation of NAC being able to increase EPO production [30].

In hyperbaric oxygen sessions, NAC has been used to reduce "toxic oxygen effects" and is still used for HBO preconditioning [31]. When administered a few days before and during HBO therapy, NAC is protective against HBO radical stress as measured by lipid peroxidation. This is a useful strategy when a prolonged treatment course is likely.

Since an up-regulation of Glutathione Synthetase is induced by repetitive exposures to higher oxygen levels, the

repetitive short normobaric oxygen sessions could well increase glutathione activity or its available amount. This needs to be considered when interpreting the acute EPO production, since an increase of haemoglobin levels has been shown using the NOP in non-healthy subjects, considered at a less than maximal efficiency or availability of glutathione.

The dose of oxygen given

Since the beginning we understood that a too high partial pressures of oxygen was responsible for a decrease of EPO (our initial results showed that hyperbaric oxygen therapy was suppressing EPO in a very significant way [5]) and of course this can be explained by the glutathione activity depletion when faced to a drastic increase of oxygen reactive species.

This phenomenon, when adequately used has been postulated to be beneficial for cancer [13]. We have also experienced that normobaric oxygen, given at too high concentrations or even too often was not as effective at increasing EPO or haemoglobin. The minimal concentration of oxygen seems to lay around 40%, raising the inspired oxygen fraction too close to 100% shows very variable results. We do not know the optimal dose or the optimal frequency and we still encourage investigations on that topic. Further confirmation of this phenomenon has recently been published [32] .These authors demonstrated that a prolonged period breathing concentrations as low as 40% oxygen can trigger increased EPO production at 24 hours after coming back to air breathing..

Conclusion

We want to draw the readers' attention to the rather fine-tuning that seems to underlay the appropriate use of the Normobaric Oxygen Paradox, in particular to be able to show a significant increase of EPO at a specific moment after one single oxygen exposure.

Reticulocyte and consequent haemoglobin increase needs several exposures at specific timeframes. Neither the optimal inspired fraction or administration schedule is known at this time. Concomitant NAC supplementation seems beneficial. Our group aims to use other experimental settings which we name: NACIO; this is the use of supplementing N-Acetyl-L-Cysteine (NAC), giving intravenous Iron (I), and Oxygen (O). We hope that this triple approach will be helpful for anaemic patients.

The optimal use of the NOP probably lies in divining the best combination of the above-mentioned factors. Jumping to conclusions when interpreting the results of experiments using suboptimal stimuli might restrain us from using a simple, inexpensive, safe and greatly beneficial phenomenon in the clinical setting. All the data reported demonstrate how hyperoxic and hypoxic states can potentially be manipulated as oxygen can be considering as a multifaceted molecule, not simply as good and bad. We have been studying steady-state

pathophysiology for more than 200 years; however, the future would seem to be in facing new paradigms, probably less easy to cope with but opening new frontiers.

KEY MESSAGES

- EPO production is likely dependent on “changes in oxygen levels” : the relative hypoxia, obtained after a period of hyperoxia, acts as a hypoxic trigger able to significantly increase EPO or hemoglobin levels.
- Further investigations to determine the optimal dose and the optimal frequency are clearly needed. Recent publications confirm that neither 100% oxygen nor increasing the difference in oxygenation (moving from normobaric hyperoxia to hypoxia) is optimal, and that oxygen can be administered too frequently.”

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Figure 1 : Intracellular explanation of the Normobaric Oxygen Paradox. Panel A: **Normoxia.** Normal cellular function. Panel B: **Hyperoxia**. During normobaric hyperoxia, reactive oxygen species stimulate GSH production (GSH synthetase); HIF-1a is continuously produced, but continuously inactivated by its binding to another protein, Von Hippel Lindau tumour-suppressor protein, and by subsequent ubiquitous metabolization by hydroxylation of proline residues. On **returning to normoxic conditions** (Panel C), all ROS's are neutralized by the increased intracellular GSH. This induces EPO gene expression similarly to hypoxia, and this situation could be called the normobaric oxygen paradox. Ub= ubiquinone. (Reprinted with permission [13])

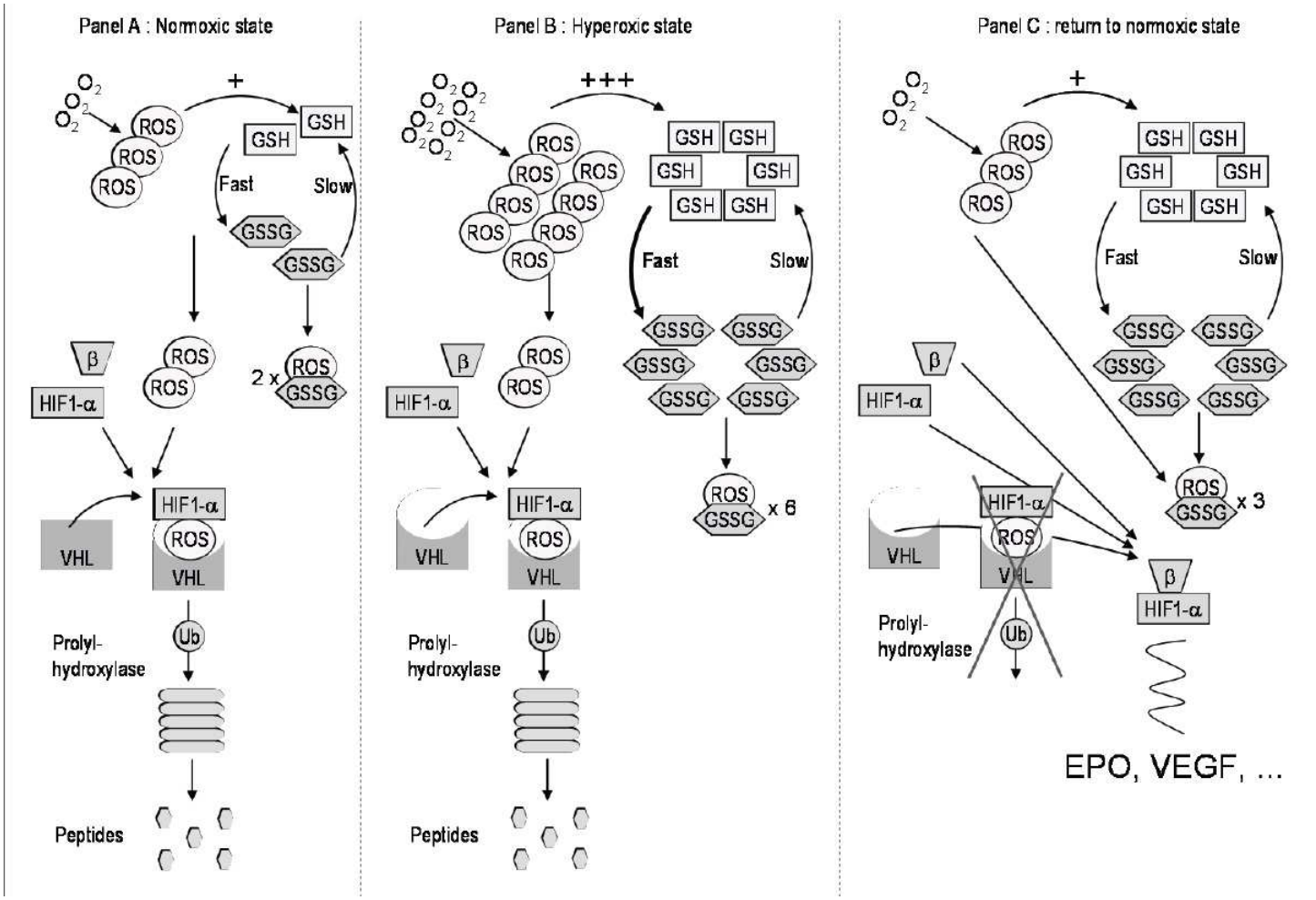

Figure 2 : Haemoglobin increase in two patients breathing pulsed oxygen. The first patient received 100% O_2 every other day with adjuvant drug therapy (Darbepoetin Alpha + IV iron) for myelofibrosis; the second patient under chemotherapy was breathing 40% O_2 three times per week with no other erythroid stimulating agents (Modified from [10]).