Title

Breath: your mitochondria will do the rest…if they are healthy!

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Abstract

Dysfunctions of the mitochondrial electron transport chain cause severe, currently untreatable, diseases in humans. A new study by Jain et al. reports increased oxygen levels in the brain of complex I-deficient mice. Reducing the O_2 levels by hypoxia, carbon monoxide or anaemia, improved the clinical phenotype and prolong the lifespan of the mouse model.

Main text

Oxygen is an extremely reactive element, with a distinguished propensity to attract electrons from other substrates, due to its high electronegativity, forming chemical bonds with almost all other elements. Through respiration, operated by the electron transfer chain (ETC), almost all eukaryotes (by means of their endosymbiont mitochondria) and aerobic bacteria use oxygen as the ultimate “sink” of electrons, converting hydrogen and oxygen atoms into harmless water. This controlled, stepwise combustion, not only allows the conversion of energy, extracted from oxidation of nutrients, into the common energy currency of the cell, ATP, but also the neutralization of the highly toxic effects of free oxygen itself. In fact, free oxygen can accept two electrons, one at a time, giving rise to highly reactive oxygen species (ROS), and its radicals, particularly the hydroxyl radical. These species react with proteins, nucleic acids, phospholipids, etc., damaging their structures and impairing their functions. Thus, like Janus, the Roman god of the passages and changes, oxygen is a double-face molecule. No life would exist on earth without it and yet it is a highly poisonous gas. The toxic effects of an excess of oxygen, produced for instance by breathing pure oxygen or gaseous mixes with high percentages of O_2, is well known, determining acute or chronic symptoms, with serious, sometimes fatal consequences on target organs, particularly the brain (Haldane, 1922), the lung and the eye. In eukaryotes, respiration is carried out within mitochondria, semi-autonomous organelles often referred to as the powerhouses of the cell because of their central role in energy metabolism. the physiological production of a low amount of ROS, an intrinsic process of normal mitochondrial respiration, acts as a signal pathway regulating mitochondrial biogenesis and bioenergetic function through a retrograde response (Butow and Avadhani, 2004), dubbed as mito-hormesis. Absence of this regulatory mechanism can indeed worsen clinical conditions due to impaired ETC (Dogan et al., 2018).

Dysfunctions of the mitochondrial ETC cause severe, currently untreatable, diseases in humans (Gorman et al., 2016). Critically, the pathogenetic mechanism of these diseases is far from clear. Defects of the mitochondrial ETC are believed to compromise the efficient utilization of oxygen in the electron flow mechanism, with consequent backlogging inversion of electron flow along the ETC. This effect amplifies the physiological leakage of electrons during respiration, causing ROS and free oxygen radicals to increase, with consequent damage of biologically relevant substrates (Murphy, 2016). This "free radical" theory has been popularized by a huge amount of experimental work and publications and has promoted the use of antioxidants as the most common pharmacological treatment of mitochondrial disease. On the other hand, absence of oxygen, or its reduction below a critical threshold, impairs the electron flow of ETC and impedes respiration and energy conversion by oxidative phosphorylation. Severe hypoxia can be produced by a number of mechanisms, including severely reduced or stop of blood flow either locally (ischemia) or in the whole organism (shock), severely reduced or arrest of breathing, severe anaemia, toxic impairment of blood oxygen-carrying haemoglobin, or by breathing air or gas mixtures with severely reduced oxygen concentration (pO_2).

In higher eukaryotes, under normoxia, the key components of the hypoxic response, i.e. the hypoxia inducible factors HIF1α and HIF2α, are rapidly hydroxylated by the prolyl hydroxylases (PHD1-3) becoming a substrate for the von Hippel-Lindau factor (vHL), an E3 ligase polyubiquitinating the HIF proteins, which are thus degraded by the proteasome (Samanta and Semenza, 2017). Contrariwise, hypoxia inhibits the activity of prolyl hydroxylases, determining refractoriness of HIF1α and HIF2α from being disposed by the vHL E3 ligase. As a consequence, HIF transcription factors accumulate, activating the "hypoxia"

genetic program, including promotion of angiogenesis and a vast repertoire of other anti-hypoxic adaptive responses that may help the survival of hypoxic tissues.

Considering the toxic effects of oxygen in conditions of failing mitochondrial respiration, in 2016 Jain et al., in Prof. Vamsi Mootha's laboratory, published important results demonstrating that a reduction in aerial pO_2 to 11% (i.e. approximately half of the concentration of normal, sea level concentration of oxygen on Earth), could determine substantial improvement in a mouse model of severe ETC complex I (cI) deficiency due to the lack of a cI structural subunit, NDUFS4 (Jain et al., 2016). This improvement included highly significant prolongation of the lifespan, substantial amelioration of the clinical symptoms, particularly those specific to the CNS, and reduction of Leigh-like disease-related lesions of the brain. Intermittent or more moderate hypoxia was ineffective to rescue the phenotype (Ferrari et al., 2017). In this new chapter of this fascinating story, Jain et al. add now some relevant mechanistic information (Jain et al., 2019). They crossed the *Ndufs4*$^{-/-}$ mice with mice lacking Phd1-3, or carrying a point mutation in the vHL, characterised by impaired HIF-related hypoxic response. They then exposed the crosses to 11% oxygen, and observed the persistence of the clinical and neuropathological improvement, therefore ruling out an essential role of the activation of the HIF program in determining the effects of breathing low-concentration oxygen.

They also showed, by exploiting two independent methodologies, that the reduced respiration rate and oxygen consumption linked to cI deficiency actually causes, when breathing normal air, a hyperoxic condition in critical tissues, which seems to be a major damaging factor in the brain in *NDUFS4*$^{-/-}$ mice. To further reinforce the evidence that hypoxia can normalize deleterious hyperoxia in *NDUFS4*$^{-/-}$ mice, they induced severe anaemia-induced hypoxia by recurrent phlebotomy and low-iron diet, or toxic hypoxia by exposing animals to

non-lethal concentrations of CO (carbon monoxide), which irreversibly dislodges oxygen from the haeme centers of haemoglobin. These findings strongly suggest that the reduced mitochondrial respiration in the *Ndufs4*$^{-/-}$ mice in normal air leads to increased pO_2 and, possibly, ROS production, in particularly in the brain. Hypoxia would thus improve the phenotype by reducing excessive local pO_2 and ROS production.

This is an important study which points to a new pathogenic mechanism for mitochondrial diseases. As a consequence of the beneficial results of hypoxia, therapeutic interventions, aimed at reducing pO_2 levels in tissues, should be beneficial to patients affected by mitochondrial disease. However, the findings by Jain and colleagues have so far been limited to a single mouse model of cI deficiency. Whether this mechanism holds true also in other models of cI-related disorders or, more generally, in other defects of the ETC is currently unknown. In addition, the methods used in this work to lower pO_2 are interesting from the experimental point of view, but it is unlikely that they can be transferred to the patients. As mentioned above, CO is a dangerous gas with a rather narrow safety window; in addition,severe anaemia would be rather risky in severely sick children.

Another conundrum that warrants additional investigation is the role of the hypoxic program in rescuing the phenotype of *Ndufs4*$^{-/-}$ mice. Previous experiments from the same authors had shown that the deletion of the vHL factor improved the growth rate and glycolytic switch of antimycin A-treated cells, a model of respiratory chain dysfunction, and also prolonged the lifespan of a vHL-less zebrafish model exposed to antimycin A. By the same token, FG4592, an inhibitor of PHD currently in clinical trial for anaemia due to kidney diseases, triggering HIF1α stabilization even in normoxic conditions was able to markedly increase the survival rate of the antimycin-treated zebrafish embryos. All these data suggest a genetic link between

the hypoxic response and the amelioration of the phenotype, but the data presented here argue against a direct role of HIF-dependent pathways *in vivo*.

Finally, the work by Jain et al. does not provide direct evidence that ROS are involved in the pathogenesis of cI-related disorders, and indeed several attempts to treat mitochondrial patients of antioxidants have given erratic results.

Unlike Janus, oxygen may well have more than two faces; we are only now starting to understand the deep consequences of altered oxygen consumption in mitochondrial dysfunction, and this will hopefully pave new avenues for treatment.

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