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Oxygenation of the newborn. The impact of one molecule on newborn lives

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Abstract: Hypoxanthine is a purine metabolite which increases during hypoxia and therefore is an indicator of this condition. Further, when hypoxanthine is oxidized to uric acid in the presence of xanthine oxidase, oxygen radicals are generated. This was the theoretical basis for suggesting and studying, beginning in the 1990s, resuscitation of newborn infants with air instead of the traditional 100% O_2. These studies demonstrated a 30% reduction in mortality when resuscitation of term and near term infants was carried out with air compared to pure oxygen. The mechanism for this is not fully understood, however the hypoxanthine –xanthine oxidase system increases oxidative stress and plays a role in regulation of the perinatal circulation. Further, hyperoxic resuscitation inhibits mitochondrial function, and one reason may be that genes involved in ATP production are down-regulated. Thus, the study of one single molecule, hypoxanthine, has contributed to the global prevention of an estimated 2–500,000 annual infant deaths.

Keywords: free radicals; hypoxanthine; newborn; oxygen; resuscitation.

Introduction

The study of one molecule, hypoxanthine, from bench to bedside, has contributed to a dramatic reduction in the use of oxygen in newborn infants both in the delivery room and beyond. It also taught us new and fundamental mechanisms related to oxidative stress not only in the newborn but in adult medicine as well. By understanding the nature of hypoxanthine the oxidative load of sick newborns has been substantially reduced the last 10–20 years contributing to a reduced morbidity in preterm infants and lower mortality in term and near term infants in need of positive pressure (PPV) at birth. It has been estimated that approximately half a million newborn lives are rescued each year after introduction of air instead of 100% oxygen for newborn resuscitation. It started in 1973 when the author was a young PhD fellow at the Perinatal Research Unit at university of Uppsala with the late professor Gösta Rooth as supervisor [1].

Biochemistry

Hypoxanthine is a naturally occurring purine metabolite with a molecular weight of 136.

Purines consist of a pyrimidine and an imidazole ring. By contrast to pyrimidine rings which in humans can be degraded completely to CO_2 and NH_2, purines are in humans degraded to uric acid which is then excreted from the body. Uric acid is predominantly formed from two sources. (1) when Guanosine monophosphate (GMP) is split into the base guanine and ribose. Guanine is deaminated to xanthine which in turn is oxidized to uric acid. (2) Deamination of Adenosine monophosphate (AMP) to Inosine monophosphate (IMP) from which the ribose unit is removed to form inosine and subsequently hypoxanthine in the presence of nucleoside phosphorylase. Hypoxanthine is also a spontaneous deamination product of adenine.

Studies with meteorites found on Earth, suggest hypoxanthine and related organic molecules, may have been formed extra-terrestrially in outer space [2].

Hypoxanthine is oxidized to xanthine and subsequently to uric acid in the presence of xanthine oxidoreductase or its superoxide radical producing version xanthine oxidase (EC 1.17.3.2). Figure 1 shows the major steps of uric acid formation. When hypoxanthine is oxidized to uric acid in two steps via xanthine by xanthine oxidase, superoxide radicals are produced. Instead of uric acid secretion, guanine and IMP can be used for recycling purposes and nucleic acid synthesis in the presence of Phosphoribosyl pyrophosphate (PRPP) Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) converts hypoxanthine into

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IMP in nucleotide salvage. The key enzyme HGPRT is lacking or deficient in the Lesch Nyhan syndrome. These patients therefore have a high uric acid level and increased oxidative stress [3, 4].

Detection

Fifty years ago hypoxanthine could be detected in body fluids by spectroscopy. However, large volumes were needed excluding this as method in human blood, especially newborns. We therefore developed a method based on the principle that oxygen is consumed when hypoxanthine is oxidized to uric acid [5]. This method was rapid and required only 0.2 mL plasma. However, it also measured half of the xanthine present. Because the ratio between xanthine and hypoxanthine is low the error by including xanthine is minor. Later HPLC methods were introduced being more reliable. High correlations between the two methods were found in plasma with a correlation coefficient of 0.96 [6].

The first study of hypoxanthine levels in human blood was published in 1975, applying the oxygen electrode method. Newborn infants with birth asphyxia had significantly higher hypoxanthine concentrations in umbilical cord than those without [7]. This finding was later confirmed by several others [8, 9].

Animal studies

Berne et al. [10] and Gerlach et al. [11] already in the early 1960s could demonstrate elevated purines in perfusion fluids of isolated organs. Cardiac hypoxia resulted in a decrease in coronary vascular resistance and a release of significant amounts of inosine and hypoxanthine from the myocardium. Based on the assumption that with hypoxia the nucleotide derivatives leave the myocardial cell as adenosine, Berne et al. proposed a hypothesis for the metabolic regulation of coronary blood flow [10].

We applied the oxygen electrode method and measured hypoxanthine in plasma of hypoxic pigs and found a linear increase with duration of hypoxia. Severity of hypoxia also affected the hypoxanthine concentration in plasma [12]. Further, in a canine model a dramatic augmentation of plasma hypoxanthine during resuscitation in endotoxin induced shock was documented [13].

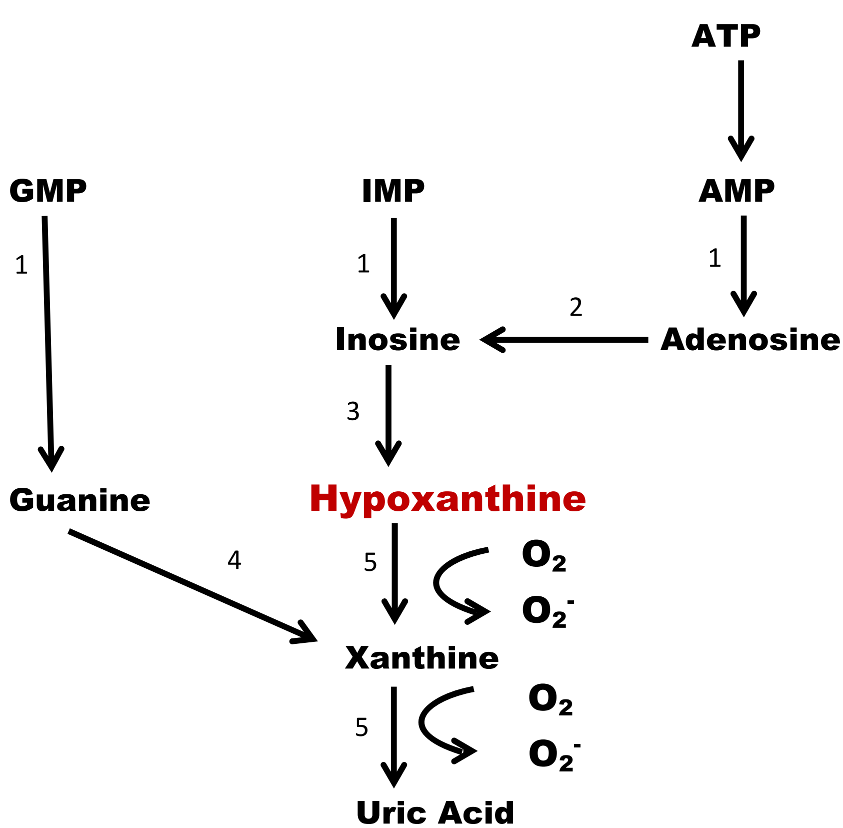

Figure 1: Formation of uric acid in the human body. Hypoxanthine is a breakdown product from AMP and IMP, and xanthine from GMP. Hypoxanthine is oxidized via xanthine to uric acid, formation superoxide radicals (O_2^-) are generated in this reaction. ATP: adenosine triphosphate, AMP: adenosine monophosphate, GMP: guanosine phosphate, IMP: inosine monophosphate. 1: nucleotidase, 2: adenosine deaminase, 3: nucleoside phosporylase, 4: guanine deaminase, 5: xanthine dehydrogenase/oxidase.

Hypoxanthine and oxidative stress

In 1969 McCord and Fridovich discovered the antioxidant superoxide dismutase (SOD) demonstrating its necessity to aerobic life [14]. One year earlier the same authors showed *in vitro* that xanthine produces oxygen free radicals when oxidized to uric acid [15]. We therefore assumed this was the case also for hypoxanthine. Based on this assumption we hypothesized that more free radicals are generated after hypoxia with high hypoxanthine levels in the tissues and body fluids, when reoxygenation is performed with supplemental oxygen. Already in 1980 we therefore questioned the practice of giving supplementary oxygen following a hypoxic episode [16].

Post mortem we also found elevated hypoxanthine in corpeous vitreum fluid of premature newborn infants who died of respiratory distress syndrome [17]. In 1988 I coined the term “oxygen radical disease of the newborn” based on the hypothesis that sick premature newborns with elevated hypoxanthine in body fluids often were given supplemental oxygen, thus increasing the risk of oxidative stress induced injury [18]. According to this hypothesis the stage could be set for free radical production simultaneously in a number of organs which often are injured in premature infants. I therefore postulated that bronchopulmonary dysplasia, retinopathy of prematurity and necrotizing enterocolitis could be triggered by the same basic mechanism, free radicals. Later we added periventricular leukomalacia to the condition named “Oxygen free radical disease of the newborn”. The concept of an “oxygen radical disease of neonatology” initiated a renewed interest for oxidative stress in neonatology and a new interest in oxygen limits and targets which paved the way for some of the large clinical studies regarding oxygenation both in the delivery room and beyond carried out around the turn of the century. By understanding the reaction of hypoxanthine it was also understood clearer that a number of factors other than oxygen itself may contribute to oxidative stress [18].

Today, the complexity of oxidative stress both in normal regulation and pathology is better understood, especially as it relates to neonatal mitochondrial oxidative stress responses to hyperoxia. Mitochondria are recipients of oxidative damage and have a propensity for oxidative self-injury that has been implicated in the pathogenesis of neonatal lung diseases. Similarly, both intrauterine growth restriction (IUGR) and macrosomia are associated with mitochondrial dysfunction and oxidative stress [19].

Moreover, the interplay between inflammation and oxidative stress in the newborn is better understood because of animal studies. Reoxygenation with 100% O_2 in a hypoxic-ischemic newborn lamb model increased the production of pro-inflammatory cytokines in the brain [20]. Transcriptomic analyses have found a number of genes to be differentially expressed in murine models of bronchopulmonary dysplasia (BPD) [21]. Epigenetic changes have also been detected both in animal models of BPD and premature infants exposed to oxygen [22, 23].

Regulation of the perinatal circulation

The hypoxanthine-xanthine oxidase system relaxes isolated constricted ductus rings from fetal lambs and constricts the pulmonary circulation of adult pigs. Clyman and co-workers could demonstrate in an *in vitro* set up that the combination of hypoxanthine and xanthine oxidase constricts the ductus arteriousis [24]. In the pulmonary circulation of adult pigs the same system lead to vasoconstriction [25, 26]. The effect was inhibited by addition of catalase, an enzyme which converts H_2O_2 to water and molecular oxygen. Since catalase, and not superoxide dismutase, inhibits the effect of the hypoxanthine-xanthine oxidase system, we believe the effect is caused by the hydroxyl radical. The hydroxyl radical stimulates prostaglandin synthesis in the vessel wall and high concentrations of prostanoids can be measured after hypoxanthine-xanthine oxidase exposure. Indomethacin inhibits the vasoactive effects of the hypoxanthine-xanthine oxidase system [24, 25].

Oxygen during resuscitation

From the early 1990s we were able to demonstrate that newborn piglets exposed to severe hypoxia could be resuscitated as well with air as with 100% O_2 [26]. Subsequent experimental studies indicated that reoxygenation with pure oxygen induces inflammation in the lung, brain, and myocardium [27–29].

The first clinical study we published in 1993 [30]. In 84 newborn infants in need of positive pressure ventilation at birth, there was no difference in short term outcome whether resuscitation was carried out with air or 100% O_2. A larger multicenter pseudo-randomized study including 609 newborn infants showed no difference in short term outcome in infants ventilated with air vs. pure oxygen. However, the 1 min Apgar score was higher and the time to first breath or cry significantly lower in the air group compared to the oxygen group [31].

Air for newborn resuscitation was then the next ten years tested out in several randomized and pseudo-randomized studies. Meta-analyses show that when compared to 100% O_2, air reduces mortality approximately 30% in term and near term newborn infants [32–35]. For newborn infants <32 weeks of gestational age the data are less clear. It seems 21% or 30% oxygen may safely be applied for infants between 28 and 32 weeks. For immature infants (≤28 weeks of gestational age) a higher initial FiO_2 seems to be needed. The optimal level is still not known [36]. However, mortality is higher in preterm newborn infants not reaching a SpO_2 of 80% within 5 min of life [37]. A recent study showed that the appropriate adjustments of FiO_2 are far more important than which initial FiO_2 is chosen [38]. Based on these findings guidelines recommend to start with 30% O_2 for infants <29 weeks GA and titrate FiO_2 according to the development of oxygen saturation measured by pulse oximetry (SpO_2). The target for titration should be SpO_2 80–85% within 5 min of life [37], and avoidance of bradycardia. A combination of hypoxemia (SpO_2<80%) and bradycardia (Heart rate<100 bpm) ≥2 min during the first 5 min of life is associated with an 18 fold increased risk of death compared to normoxemia and heart rate >100 bpm [39].

Mechanisms

Mitochondria are a major source of ROS production, easily surpassing cytosolic ROS sources [40, 41]. Mitochondrial ROS include O_2^- produced by complexes I and III of the electron transport chain, and H_2O_2 generated through the dismutation of O_2^- by mitochondrial superoxide dismutase (MnSOD). Hypoxia leads to elevation of Krebs cycle intermediates, including α-ketoglutarate, succinate, and fumarate. In a study on hypoxic newborn piglets we demonstrated a more rapid normalization of these metabolites if reoxygenation was carried out with air instead of 100% oxygen. This indicated that mitochondrial function is impaired by applying pure oxygen during reoxygenation [42]. In addition, succinate creates the so-called reverse electron transport from Complex –II to Complex-I, which generates higher reactive oxygen species (ROS) compared to the conventional forward electron transport from Complex-I to Complex-II [43, 44].

Studies in hypoxic newborn mice show that reoxygenation with 100% oxygen inhibits enzymes of all five complexes belonging to the electron transport system [45]. Altogether these findings indicate that reoxygenation with 100% O_2 inhibits or reduces ATP production, and this may explain, at least partly, the lower mortality in newborn infants resuscitated with air instead of 100% O_2. Further, long term oxygen exposure (14 days) of newborn mice trigger genomic and epigenetic changes which we don't know are permanent or not and how this influences outcome [22, 46].

Potential therapeutic principles

The most important consequence of these findings is the understanding that an effective therapy in many circumstances is to substitute pure oxygen with air. Unfortunately, antioxidant therapy to prevent newborn disease has not been as successful as expected. SOD has minor effects only in reducing pulmonary disease of premature infants [47]. However, Farrow et al. in a newborn lamb model with primary pulmonary hypertension (PPHN) found that intra-tracheal recombinant super oxide dismutase (rh SOD) diminished reactive oxygen species production [48]. The same group also found that rhSOD rapidly increased oxygenation and reduced vasoconstriction in newborn lambs with PPHN [49].

In a review Ofman and Tipple outline major barriers to effective anti-oxidant therapies: lack of compartment and target specificity, limited bioavailability, timing of therapy, and genetic variability [50]. Endogenous antioxidant enzymes are highly localized and perform specific functions in redox biochemistry. Systemic or even organ-specific administration of antioxidants may not reach the cells or organelles where hyperoxia induces injury. In addition, individual organs have variable oxygen consumption and respond differently to hypoxia and hyperoxia [39, 50]. This is evident in the NeOProM trials, where lower SpO_2 targets were associated with NEC and mortality while higher SpO_2 targets were associated with ROP [51]. In the future, a bioengineered heme-containing protein, OMX-CV, may have a therapeutic potential. This substance has a 10-fold higher affinity for oxygen than hemoglobin has been shown in juvenile lambs to selectively deliver oxygen to hypoxic tissues, but not those at physiologic oxygen tension [52]. Thioredoxin is an antioxidant enzyme that catalyzes the reduction of oxidized cysteine residues, playing an important role in reducing protein disulfide bonds. Alterations in thioredoxin and thioredoxin reductase, have been shown to affect susceptibility to hyperoxic lung injury [53]. Drugs such as auranofin and aurothioglucose inhibit thioredoxin reductase, and have been shown to protect mice from hyperoxic lung injury. Similarly, melatonin is a neurohormone that has been shown to mitigate hyperoxic lung injury in rats as well as hypoxic ischemic encephalopathy in pigs [39, 53].

Discussion

The study of hypoxanthine, from bench to bedside, has during 40–50 years changed clinical practice. Cochrane reviews have demonstrated a 4.9% absolute reduction of mortality, 16.2 Vs 11.3%, when newborns are resuscitated with air instead of 100% O_2 [32]. This indicates that 196,000 deaths out of 4 million estimated term or near term newborn infants with intrapartum related events (birth asphyxia) at the turn of the century may be prevented by using air instead of 100% O_2 for resuscitation (Typical RR 0.71 (0.54, 0.94), typical RD-0.05 (−0.08, −0.01), NNT 20). Importantly, no increase in hypoxic ischemic encephalopathy has been found in infants resuscitated with air instead of pure oxygen [54].

Further, room air resuscitation paved the way for resuscitation programs without pure oxygen, such as Helping Babies Breathe (HBB). This program includes newborn infants who previously were classified as “fresh stillbirths” and consequently were not even tried to be ventilated [55]. Carlo et al. demonstrated that active resuscitation with bag and mask air ventilation of such infants may rescue 30% (RR 0.69; 95% CI, 0.54 to 0.88; p=0.003) [56]. Consequently, out of approximately 1 million annual fresh stillbirths, 300,000 may be resuscitable by bag and mask air-ventilation. Air resuscitation therefore potentially may annually prevent approximately $196{,}000 + 300{,}000 \approx 500{,}000$ of newborn deaths and fresh stillbirths.

The oxidative load of both term and preterm infants in need of acute and long term therapy has been dramatically lowered during the last decades. The study of one molecule, hypoxanthine, showed that oxygen is more toxic than previously believed [57]. This has changed the world of neonatology. By applying less oxygen to newborn infants the prevention of half a million deaths may be achieved globally each year.

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