Redox Biology 12 (2017) 674–681

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Review article

Oxygen and oxidative stress in the perinatal period

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ARTICLE INFO

Keywords:
Oxygen
Oxidative stress
Ischemia-reperfusion
High-risk pregnancy
Newborn
Biomarkers

ABSTRACT

Fetal life evolves in a hypoxic environment. Changes in the oxygen content *in utero* caused by conditions such as pre-eclampsia or type I diabetes or by oxygen supplementation to the mother lead to increased free radical production and correlate with perinatal outcomes.

In the fetal-to-neonatal transition asphyxia is characterized by intermittent periods of hypoxia ischemia that may evolve to hypoxic ischemic encephalopathy associated with neurocognitive, motor, and neurosensorial impairment. Free radicals generated upon reoxygenation may notably increase brain damage. Hence, clinical trials have shown that the use of 100% oxygen given with positive pressure in the airways of the newborn infant during resuscitation causes more oxidative stress than using air, and increases mortality.

Preterm infants are endowed with an immature lung and antioxidant system. Clinical stabilization of preterm infants after birth frequently requires positive pressure ventilation with a gas admixture that contains oxygen to achieve a normal heart rate and arterial oxygen saturation. In randomized controlled trials the use high oxygen concentrations (90% to 100%) has caused more oxidative stress and clinical complications that the use of lower oxygen concentrations (30–60%). A correlation between the amount of oxygen received during resuscitation and the level of biomarkers of oxidative stress and clinical outcomes was established. Thus, based on clinical outcomes and analytical results of oxidative stress biomarkers relevant changes were introduced in the resuscitation policies. However, it should be underscored that analysis of oxidative stress biomarkers in biofluids has only been used in experimental and clinical research but not in clinical routine. The complexity of the technical procedures, lack of automation, and cost of these determinations have hindered the routine use of biomarkers in the clinical setting. Overcoming these technical and economical difficulties constitutes a challenge for the immediate future since accurate evaluation of oxidative stress would contribute to improve the quality of care of our neonatal patients.

1. Introduction

1.1. Aerobic metabolism and oxidative stress

Oxygen is the final acceptor of highly energized electrons generated at different metabolic processes being the most relevant the oxidases' activity (xanthine oxido-reductase; NADPH oxidase), nitric oxide synthase (NOS), and the mitochondrial oxidative phosphorylation process. Under physiologic conditions, a small percentage of the total oxygen metabolized during aerobic metabolism is incompletely reduced leading to the formation reactive oxygen species (ROS). The most common ROS in human biology result for the reduction of oxygen

Abbreviations: AA, arachidonic acid; AF, amniotic fluid; BPD, bronchopulmonary dysplasia; CAT, catalase; CS, cesarean section; DHA, docosahexanoic acid; DR, delivery room; EPO, erythropoietin; FiO_2, oxygen inspiratory fraction 0.21–1.0); GC-MS/MS, gas chromatography coupled to tandem mass spectrometry; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; HIF, hypoxia inducible factor; HPLC-MS/MS, high-performance liquid chromatography coupled to tandem mass spectrometry; IsoFs, Isofurans; IsoPs, Isoprostanes; IUGR, intrauterine growth retardation; kPa, kilopascal; NAC, N-acetyl-cysteine; NFκB, nuclear factor kappa B; NADPH, phosphorylated nicotine adenine dinucleotide; NOS, nitric oxide synthase; NeuroPs, Neuroprostanes; NeuroFs, Neurofurans; P_aO_2/p_aO_2, partial pressure of oxygen in arterial blood (mmHg); $PIVO_2$, intervillous (placenta) partial pressure of oxygen (mmHg); PGF, placental growth factor; ppO, partial pressure of oxygen (mmHg); RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; SpO_2, arterial oxygen saturation (expressed in %); VEGF, vascular endothelial growth factor; XD, xanthine dehydrogenase; XO, xanthine oxidase

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http://dx.doi.org/10.1016/j.redox.2017.03.011
Received 23 January 2017; Received in revised form 12 February 2017; Accepted 9 March 2017
Available online 12 March 2017

with just one electron to form superoxide anion ($\bullet O_2^-$). In addition, the reduction of oxygen with 2 or 3 electrons leads to the formation of hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH), respectively. In addition, nitric oxide (NO•) may combine with oxygen free radicals especially anion superoxide to conform peroxynitrite ($ONOO^-$) a reactive nitrogen species (RNS). ROS and RNS have short half-lives and react with nearby molecules such as proteins, DNA, RNA, glucids or free fatty acids begetting them as free radicals and altering their structure and/or function. In the presence of transition metals especially iron (Fe^{+2}) generation of hydroxyl radical is highly enhanced (Fenton chemistry). However, ROS and especially hydrogen peroxide can also act as cell-signaling molecules and regulate cell redox processes. Oxidative stress is broadly referred to as an imbalance between the generation of ROS and RNS and their clearance by the antioxidant defense system and has been associated with placental oxidative disorders and immune disturbances and newborn conditions [1–3].

Human life *in utero* elapses in an environment that is relatively hypoxic as compared to the *ex utero*. However, oxygen availability is provided by exquisite adaptive mechanisms that allow for an extraordinary growth and development that exceeds any other period of life. Maternal conditions during pregnancy may cause fetal hypoxia. Chronic hypoxia is caused by vascular or metabolic alterations in the mother such as preeclampsia, obesity or diabetes. Hypoxic fetuses are at higher risk of developing oxidative and nitrosative stress that may be determining for their *in utero* development and postnatal development [4].

Acute hypoxia leading to asphyxia is characterized by profound acidosis, base deficit and lactacidemia. Given this situation resuscitation maneuvers immediately after birth are indispensable for patient's survival; however, mechanisms inherent to ischemia-reoxygenation will inevitably increase initial damage [5]. Therefore, interventions to avoid reoxygenation damage such as reducing the inspiratory fraction of oxygen (FiO_2) in the delivery room (DR) have been advocated for resuscitation of asphyctic neonates and for preterm infants with immature lungs, surfactant production and antioxidant defense system [6,7].

1.2. Biomarkers of oxidative stress and clinical application

Biomarkers could be defined as metabolites that can be objectively measured in biofluids (plasma, urine, spinal fluid, etc.) in the laboratory and accurately reflect either normal biological or pathological processes or response to pharmacologic interventions. In the clinical setting they acquire relevance when capable of assessing response to specifically tailored treatments or are able to predict short and/or long term outcomes [8]. Sensitivity and specificity define the accuracy of a biomarker to identify and quantify changes in biomolecules available in easily accessible biofluids (plasma, urine, amniotic fluid, etc.). Furthermore, the ideal biomarker should remain stable during storing, require small volume, preferably non-invasively obtained (urine), easily determined by automatized methods, reproducible, quantitatively expressed results, and preferably inexpensive [9]. The newborn infant is at high risk for oxygen free radical derived conditions [10]. Many of the most relevant pathologies associated with prematurity such as birth hypoxia, retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD) or intraventricular hemorrhage (IVH) have been associated with the use of oxygen supplementation and the immaturity of the antioxidant defense system in the postnatal period [11,12]. However, the newborn infant and especially the very preterm have unique physical characteristics that limit the accessibility to blood vessels and the volume of blood that can be drawn for analytical purposes. Moreover, ethical considerations recommend limiting painful procedures. Both gas chromatography and high performance liquid chromatography coupled to tandem mass spectrometry (GC-MS/MS, HPLC-MS/MS) have been widely used to obtain snapshots of the oxidant status in plasma or serum of the newborn infant [13]. However, more recently repeated urine analysis have allowed to non-invasively monitor changes over time of the oxidant status after specific therapeutic interventions [14,15].

Although highly reliable methods have been put forward enabling multi-analyte detection in different biofluids the use of biomarkers of oxidative stress in neonatology has been almost confined to clinical research. The complex methodology requiring sophisticated equipment and highly specialized technicians, elevated cost, delay in providing results, unavailability round the clock, and the difficulty in interpreting data from a clinical point of view has limited the use of oxidative stress biomarkers to experimental and clinical research. However, the results of oxidative stress biomarkers has been crucial to support changes in the guidelines of newborn resuscitation that now recommend the use of use of room air instead of pure oxygen in asphyxiated term infants [16]. Moreover, the amount of oxygen provided during the stabilization of preterm infants correlates with the level of plasma and/or urinary biomarkers of oxidative stress and with the development of BPD [15,17–19]. These findings have notably influence the restriction of oxygen supplementation in the delivery room in preterm infants [17]. Further clinical application of oxidative stress biomarkers relates to the studies of Winterbourne et al. that have found increased levels of glutathione sulfonamide (GSA) in bronchoalveolar lavage fluid in preterm infants who later developed lung infection or BPD [20–22]. Using a similar analytical methodology, our group found increased

Table 1
Analytical biomarkers used for the assessment of oxidative stress in clinical research in the perinatal period and most reliable techniques employed for its measurement.

Oxidative biomarkers	Target biomolecule	Modification	Biological sampling	Analytical method
Glutathione (GSH/GSSG ratio)	Antioxidants	General Redox Status	Total Blood	LC-MS/MS
o-Tyrosine (o-Tyr/Phe ratio)	Proteins		Urine	HPLC-MS/MS
m-Tyrosine (m-Tyr/Phe ratio)	Proteins		Urine	HPLC-MS/MS
3N2-Tyrosine	Proteins	Tyrosine nitration	Urine	HPLC-MS/MS
8OHdG (8OHdG/2dG ratio)	DNA		Urine/Plasma/Serum/CSF/AF	HPLC-MS/MS
F2-IsoPs	DNA	DNA oxidation	Urine/Plasma/Serum/CSF/AF	GC-MS/MS; HPLC-MS/MS
D2/F2-ISoPs	DNA	DNA oxidation	Urine/Plasma/Serum/CSF/AF	GC-MS/MS; HPLC-MS/MS
IsoFs	DNA	DNA oxidation	Urine/Plasma/Serum/CSF/AF	GC-MS/MS; HPLC-MS/MS
NeuPs	DNA	DHA Peroxidation	Urine/Plasma/CSF/AF	GC-MS/MS; HPLC-MS/MS
NeuFs	DNA	DHA Peroxidation	Urine/Plasma/CSF/AF	GC-MS/MS; HPLC-MS/MS

Abbreviations: GSH: reduced glutathione; GSSG: oxidized glutathione; o-Tyr: ortho-tyrosine; m-Tyr: meta-tyrosine; 3N2-Tyrosine: 3-nitrotyrosine; 8OHdG: 8-hydroxi-2′-deoxiguanosine; 2dG: 2′-deoxiguanosine; IsoPs: isoprostanes; IsoFs: isofurans; NeuPs: Neuroprostanes; NeuFs: neurofurans; AA: arachidonic acid; DHA: docosahexanoic acid; CSF: cerebral spinal fluid; AF: amniotic fluid; LC: liquid chromatography: GC: gas chromatography; MS/MS: tandem mass spectrometry.