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Oxidative Stress of Newborn

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1. Introduction

Free radicals are highly reactive molecules containing one or more unpaired electrons. They donate or abstract electrons from other molecules in an attempt to pair their electrons and generate a more stable species. Oxygen-derived reactants collectively termed reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) are normally produced in living organisms. When produced in excess, they are important mediators of cell and tissue injury [Halliwell, B. (1999), Fridovich, I. (1998), Gitto, E. et al. (2002)]. The resulting damage is referred to as oxidative stress. Free radicals are highly unstable and several enzymes and small-molecular-weight molecules with antioxidant capabilities protect against them [Halliwell, B. (1992)]; these protective molecules are part of the antioxidative defence system. There is a critical balance between free radical generation and antioxidant defences. Free radical reactions lead to the oxidation of lipids, proteins, polysaccharides and to DNA damage (fragmentation, base modifications and strand breaks); as a consequence, radicals have a wide range of biologically toxic effects [Saugstad, OD. (1996), Sarker, AH. et al. (1995)]. The generation of both ROS and RNS are summarised in figure 1.

Newborns and especially pre-term infants are probably more prone to oxidative stress than are children and young adults. There are some special reasons for this. These infants very often 1) are exposed to high oxygen concentrations, 2) have infections or inflammation, 3) have reduced antioxidant defence, and 4) have free iron which enhances the Fenton reaction leading to production of highly toxic hydroxyl radicals [Saugstad, OD. (2003, 2005)]. The Fenton reaction describes the interaction of hydrogen peroxide with a transition metal resulting in the generation of the highly toxic hydroxyl radical. Oxidative stress has been postulated to be implicated in several newborn conditions and, in 1988, SAUGSTAD [Saugstad, OD. (1988)] coined the phrase ''oxygen radical diseases of neonatology''. The idea contends that oxidative stress affects different organs, often simultaneously, giving rise to different signs according to the organ most affected. He included bronchopulmonary dysplasia/chronic lung disease, retinopathy of prematurity and necrotising enterocolitis in this category. Later, it became clear that free radicals are also involved in periventricular

leukomalacia [Haynes, RL. et al. (2003)] as well as in regulating the ductus arteriosus and pulmonary circulation [Clyman, RI. et al. (1989), Archer, SL. et al. (1989), Sanderud, J. et al. (1993)]. If the concept of ''oxygen radical diseases in neonatology'' is correct, it means that the conditions mentioned are not different diseases but belong to the same frequently have higher plasma levels of non-transferrin-bound iron and higher erythrocyte free iron than adults [Ogihara, T. et al. (1996)].

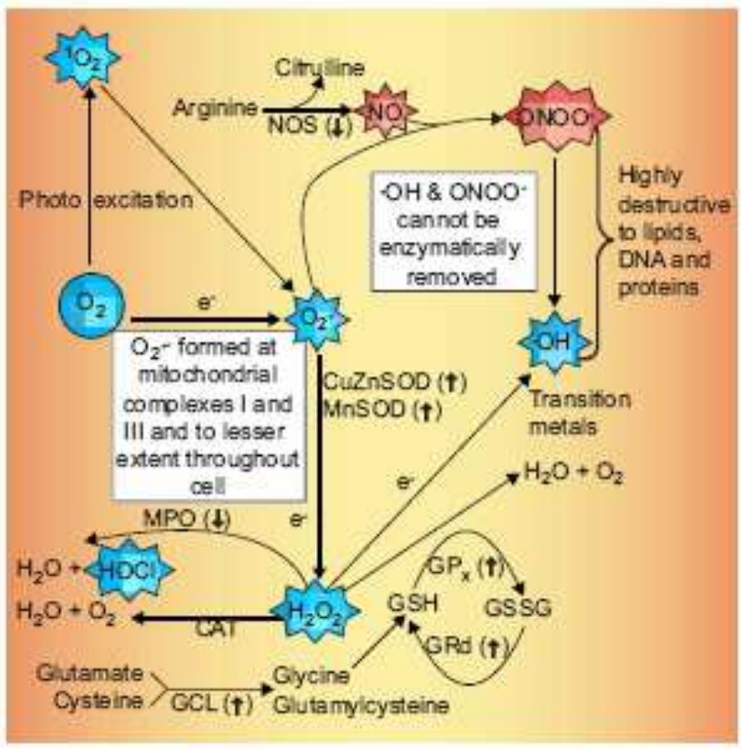

Fig. 1. Summary of the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS).

2. Oxidative stress in pregnancy, in pre-eclampsia and at parturition

Pregnancy is a physiological state accompanied by a high metabolic demand and elevated requirements for tissue oxygen. This increased oxygen demand augments the rate of production of ROS and even women with normal pregnancies experience increased oxidative stress and lipid peroxidation relative to age-matched, non pregnant women. Several studies have shown that the antioxidative defense system is altered during pregnancy. Circulating levels of lipid peroxides increase significantly in the maternal circulation when a woman becomes pregnant. Various antioxidants, however, including vitamin E, ceruloplasmin, erythrocyte thiols and iron-binding capacity also increase. Several of these elevate progressively with advancing gestation, while serum iron concentrations progressively decrease. While there is a gradual favoring of antioxidant activity over oxidation during normal pregnancy, there is an insufficient increase in antioxidants to offset the rise in free radical generation. In contrast to the low-molecular weight antioxidants, the

activity of an important family of antioxidative enzymes, the superoxide dismutases (SOD), are depressed in the blood of pregnant women [Wisdom, SJ. et al. (1991)]. In addition, Walsh and Wang [Walsh, SW. & Wang, Y. (1993)] reported a deficiency in another antioxidative enzyme, glutathione peroxidase (GPx), during pregnancy. GPx is an important antioxidant enzyme present in virtually all tissues. The enzyme limits the accumulation of lipid peroxides and utilizes reduced glutathione (GSH) as its cofactor to convert lipid peroxides into relatively harmless hydroxylated fatty acids, water and glutathione disulfide. Given these actions, it might be expected that a deficiency in this enzyme may lead to elevated oxidative stress during pregnancy. The placenta is a major source of oxidative stress during pregnancy. It is rich in polyunsaturated fatty acids, and the placenta is an abundant source of lipid peroxides which are secreted into the maternal circulation. In normal pregnancy, placental lipid production is believed to be kept under control by placental antioxidant enzymes [Walsh, SW. et al. (1993)]. Major antioxidant enzymes such as SOD, catalase (CAT), GPx, glutathione reductase, glutathione S-transferase and glucose-6-phosphate dehydrogenase are all present in the placenta. In the normal placenta, the activities of SOD and CAT increase as gestation progresses, while the activity of GPx is diminished. On the other hand, placental production of lipid peroxides progressively drop as normal gestation advances, most likely because of the elevated activities of SOD and CAT. Thus, in normal pregnancy, placental antioxidant defenses are considered sufficient to control lipid peroxidation. Pre-eclampsia is a multisystem disorder unique to human pregnancy. It is a complication in 5–10% of pregnancies and remains a leading cause of maternal and neonatal mortality and morbidity. This human disorder is a leading cause of premature delivery and intrauterine fetal growth retardation (IUGR). Pre-eclampsia is usually diagnosed in late pregnancy because of increased blood pressure and proteinuria with the symptoms of pre-eclampsia typically disappearing shortly after delivery of the placenta. A significant rise in lipid peroxidation levels in the placenta of pre-eclampsia has been suggested [Walsh, SW. et al. (2000), Hubel, CA. (1999), Gupta, S. et al. (2005), Vanderlelie, J. et al. (2005), Atamer, Y. et al. (2005)]. Several lines of evidence support this assumption, including increased lipid peroxidation products, elevated nitrotyrosine immunostaining and reduced antioxidant enzyme activities in preeclamptic placentas. In a case control study, Vanderlelie et al. [Vanderlelie, J. et al. (2005)] measured tissue levels of SOD, GPx and lipid peroxidation in placental samples from women with normal pregnancies (18 women) and with pre-eclampsia (20 women). Placental tissue homogenates from pre-eclamptic patients contained significantly higher levels of lipid peroxides [malondialdehyde (MDA) and 4-hydroxy-2 (E)- nonenal; 20.68 versus 5.33 mm/mg protein], whereas there were significantly lower levels of SOD (2.02 versus 2.48 U/ mg protein) and of GPx (11.50 versus 17.33 mmol/min/mg) than in control placentas. These finding are consistent with a limited enzymatic antioxidant capacity and elevated breakdown of lipids in placental tissue of women suffering from pre-eclampsia. Increased levels of thromboxane and lipid peroxides associated with a loss of GPx activity was also reported in placentas from pre-eclamptic patients compared with those from normal pregnancies [Walsh, SW. & Wang, Y. (1993)]. In parallel, the in vitro production of lipid peroxides and thromboxane is augmented in both trophoblast cells and villous tissues from women with pre-eclampsia [Walsh, SW. & Wang, Y. (1995)]. Furthermore, production of 8-iso-PGF2a and MDA (a

lipid peroxide metabolite), as measured by levels in the medium, is higher for pre-eclamptic placental tissue explants than for normal placental explants [Walsh, SW. et al. (2000)]. Collectively, the data provide convincing evidence that oxidative stress and especially lipid peroxidation are abnormally increased in the placentas of pre eclamptic women. Many et al. [Many, A. et al. (2000)] found particularly intensive immunoreactivity for nitrotyrosine in invasive cytotrophoblasts in placental biopsies and vascular endothelium in the floating villi obtained from women with pre-eclampsia. The presence of nitrotyrosine is suggestive of damage caused by peroxynitrite, a potent nitrosative agent [Beckman, JS. & Koppenol, WH. (1996)]. Overall, the findings of nitrotyrosine residues in the cellular components of pre-eclamptic placentas may reflect increased production of the superoxide anion radical, as it couples with nitric oxide to generate peroxynitrite. Placental generation of ROS and reactive nitrogen species (RNS) in pre-eclampsia might be facilitated by a reduction in local antioxidant defense, although it is not clear whether this reduced antioxidant defense is part of the problem or secondary to free radical damage. The activities of placental SOD and glucose 6 phosphate-dehydrogenase are reduced in pre-eclampsia compared to placentas from normal pregnancy [Poranen, AK. et al. (1996)]. Moreover, the activities and mRNA expression of Cu/ZnSOD and GPx, and tissue levels of vitamin E are significantly lower in placental tissues from pre-eclampsia than from normal pregnancy [Wang, Y. & Walsh, SW. (1996)]. In summary, there appears to be an increment in ROS generation in the placenta of pre-eclamptic women. There is also evidence for increased nitrotyrosine residue formation in the pre eclamptic placenta suggestive of peroxynitrite formation, perhaps arising from local NO production coupled with increased generation of superoxide anion radical and either regionally decreased or inadequate SOD. The transition from fetal to neonatal life at birth includes acute and complex physiologic changes. The delivery of the fetus from an intrauterine relatively hypoxic environment with a PO2 of 20–25 mmHg to an extrauterine normoxic environment with a PO2 of 100 mmHg increases oxidative stress. This four to fivefold rise in oxygen tension is believed to induce a greater production of ROS [Shoji, H. & Koletzko, B. (2007)]. In addition, labor and childbirth may be associated with periods of both hypoxia and oxidative stress for the newborn, while neonatal plasma is relatively deficient in antioxidants. Several investigators studied the relationship between the oxidative state of the mother and the newborn at the moment of birth. Arguelles et al. [Auguelles, S. et al. (2006)] measured oxidative stress markers [carbonyl groups, lipid peroxides and total antioxidant capacity (TAC)] and found a good correlation between the oxidative status of the mother and of the neonate, with higher oxidative stress correlating with an even higher oxidative stress of the newborn based on measurements in umbilical cord blood. They also report that smoking mothers and their newborns had a higher concentration of the carbonyl groups, lipid peroxides and a lower TAC. Term labor is typically associated with oxidative stress for the neonate, but there is no difference between the degree of fetal oxidative stress in vaginal delivery and cesarean section [Fogel, I. et al. (2005), Hracsko, Z. et al. (2007)]. It is unclear, however, whether oxidative stress is related to the delivery itself or whether it reflects a pre-existing fetal oxidative status. Lauries et al. [Laurie, S. et al. (2007)] demonstrated that distressed fetuses delivered by emergency cesarean exhibited increased MDA concentrations, a parameter indicative of oxidative damage, and an enhanced GPx activity in amniotic fluid and

umbilical cord blood compared to non distressed fetuses delivered by elective cesarean section. This is probably an indication of higher fetal oxidative stress.

2.1. Oxidative stress in neonatal diseases

Perinatal asphyxia is an insult to the fetus or newborn resulting from a lack of oxygen (hypoxia) or a reduced perfusion (ischemia) in various organs. While virtually every organ of the body is affected by asphyxia leading to multiorgan failure, the most severe insult occurs in the central nervous system, which also lacks many repair processes [Scher, M. (2001)]. The mechanism of cellular injury after hypoxia or ischemia is poorly understood, but is probably mediated by an excess release of neurotransmitters, generation of ROS/RNS and the initiation of lipid peroxidation which, in turn, leads to a cascade of damaging events [Harris, ED. (1992)].

At the cellular level, cerebral hypoxia–ischemia sets in motion a series of biochemical events commencing in a shift from oxidative to anaerobic metabolism; this leads to an accumulation of NADH, FADH, lactic acid and H+ ions [Palmer, C. et al. (1990)]. If the asphyxic insult persists, the fetus is unable to maintain circulatory centralization, cardiac output and cerebral perfusion falls. Owing to the acute reduction in its oxygen supply, oxidative phosphorylation and ATP production in the brain are diminished [Yager, JY. et al. (1992), Berger, R. et al. (1994)]. As a result, the Na+/K+ pump in cell membranes are deprived of the required energy to maintain ionic gradients. With a reduced membrane potential, increased numbers of calcium ions flow through voltage-dependent ion channels, down an extreme extra-intracellular concentration gradient, into the cell. Intracellular accumulation of Na+ and Cl) ions leads to swelling of the cells as water enters by osmosis (cytotoxic cell edema) [Vannucci, RC. et al. (1993)]. This damage is thought to be caused by the post ischemic production of oxygen radicals, synthesis of NO, inflammatory reactions and an imbalance between excitatory and inhibitory neurotransmitter systems. Part of the secondary neuronal cell damage may be caused by induction of a well-known cellular suicide program referred to as apoptosis [Berger, R. & Garnier, J. (2000)]. Production of reactive species in the early reperfusion phase plays a substantial role in the resulting brain cell damage. Among the toxicants generated are the superoxide anion radical (O2 •)) and hydrogen peroxide (H2O2). The latter agent can be converted to the highly reactive hydroxyl radical by transition metals, in particular free iron, ultimately leading to lipid peroxidation of the brain cell membranes as well as damage to other macromolecules [Halliwell, B. & Gutteridge, JC. (1990)].

Recent studies reported increased intra-erythrocyte free iron levels in infants with asphyxia [Buonocore, G. et al. (1998)]. Iron may be released from hemoglobin in erythrocytes as result of oxidative stress [Ferrali, M. et al. (1992)]. As the erythrocyte is a target of extracellular free radicals, free iron release may be followed by extracellular oxidative stress caused by O2 •) generation because of phagocyte activation [Buonocore, G. et al. (1994)]. Intra-erythrocyte free iron concentrations appear to be a reliable marker of cell oxidative stress and an indicator of the risk of oxidative injury in other tissues. Increased production of free radicals including the O2 •) and NO induces oxidative stress in the placenta by formation of the pro-oxidant ONOO); this reactant is formed when O2 •) couples with NO• [Crow, JP. & Beckman, JS. (1996), Szabo, C. & Oshima, H. (1997)]. ONOO) is cytotoxic due to a number of independent mechanisms including the initiation of lipid peroxidation, the inactivation of a

variety of enzymes (most notably, mitochondrial respiratory enzymes) and membrane pumps [Crow, JP. & Beckman, JS. (1995)] and depletion of GSH [Phelps, DT. et al. (1995)]. Moreover, ONOO) causes DNA damage [Inoue, S. & Kawanishi, S. (1995), Salgo, MG. et al. (1995)] resulting in the activation of the nuclear enzyme poly (ADP-ribose) synthetase, depletion of NAD and ATP, and ultimately leading to cell death [Szabo, C. et al. (1997)]. The higher levels of free radical production associated with repetitive ischemia likely reflect differences in oxygen availability during these events. During repetitive ischemia, the availability of oxygen to the fetus for intermittent periods of reperfusion facilitates free radical production. With the increased availability of oxygen, oxidative reactions rather than reductive reactions are favored. Hyperoxic exposure itself, although essential for promoting survival of infants with RDS, induces excessive production of ROS in the respiratory system. There exist, however, several potential causes of intracellular and extracellular oxidant stress in the preterm newborns with RDS. The high inspiratory concentrations of oxygen required to achieve adequate arterial oxygenation, pro-oxidant drugs and infections or extrapulmonary inflammation can all promote ROS accumulation and the utilization and depletion of antioxidative agents [Kothecha, S. (2000)].

In experimental models of respiratory distress, the specific targets of a hyperoxic insult to the lung are the vascular endothelial cells and the epithelial cells of the alveoli. ROS induce ultrastructural changes in the cytoplasm of pulmonary capillary endothelial cells and cause focal hypertrophy and altered metabolic activity. Thus, increased oxidative stress accompanied by reduced endogenous antioxidant defenses may play a role in the pathogenesis of a number of inflammatory pulmonary diseases including respiratory distress in the newborn [Bhandari, V. et al. (2000), Ikegami, M. et al. (2000)]. A deficit in the precise balance between exposure to oxidants and endogenous antioxidants obviously leads to elevated oxidative damage. The molecular damage caused by free radicals and related reactants appear to be involved in the pathogenesis of a growing number of diseases, including RDS of the newborn [Lamb, NJ. et al. (1999), Huertas, JR. et al. (1998)]. When phagocytes such as neutrophils are stimulated by microorganisms or other means, they are activated and increase their oxidative metabolism; as a result, toxic oxygen derivatives, i.e. ROS, are formed. If these oxygen based products are not inactivated, their high chemical reactivity leads to damage to a variety of cellular macromolecules including proteins, carbohydrates, lipids and nucleic acid. This results in cell injury and may induce respiratory cell death [Esteban, J. et al. (1999)]. Under these conditions, a surfactant deficiency may be aggravated by the inactivation of the small amount of endogenous surfactant that is produced [Boda, D. et al. (1998)]. Furthermore, if exogenous surfactant is given, it may also be destroyed [Ikegami, M. et al. (2000), Huertas, JR. et al. (1998)]. Reactive oxygen species also have been implicated in the molecular damage seen in the bronchoalveolar lavage (BAL) fluid of patients with RDS [Banks, BA. et al. (1998), Dellinger, RP. (1999)]. This assertion is supported by several findings; H2O2 is detected in the expired air of RDS patients, and myeloperoxidase (MPO) and oxidized-1-antitrypsin have been found in BAL fluid. Moreover, increased plasma lipid peroxidation products have been measured in critically ill patients and in patients with sepsis and at risk of developing RDS. Also, evidence of augmented levels of oxidized lipids and proteins have been found in the plasma of patients with RDS. Elevated levels of ROS also have been implicated in the molecular damage seen in the BAL fluid of patients with RDS. BAL fluid normally contains a large amount of the antioxidant GSH; however, in patients with RDS this is mostly in the oxidized

form [Janssen, YMW. et al. (1998)]. Consistent with this, oxidative inactivation of 1-antiprotease also has been observed in RDS. Elevated concentrations of xanthine and hypoxanthine are present in the plasma and BAL fluid of patients with RDS and are a potential source of ROS in the presence of exogenously added xanthine oxidase. Also, elevated concentrations of orthotyrosine and metatyrosine in BAL fluid protein imply the formation of the damaging •OH in the lungs of these patients, as orthotyrosine and metatyrosine are isomers of tyrosine thought to be formed exclusively by aromatic hydroxylation of phenylalanine by •OH [Kim, K. et al. (1999)]. Chlorotyrosine and nitrotyrosine also have been found in BAL fluid from patients with RDS. Increased concentrations of chlorotyrosine residues in BAL fluid proteins from patients with RDS indicate hypochlorous acid (HClO) production by the activated inflammatory cells in the lungs of these patients. Chlorotyrosine is formed by HClO-dependent chlorination of paratyrosine. HClO is a damaging oxidant formed from H2O2 and chloride ions by the enzyme MPO, present in activated inflammatory cells. HClO has been implicated as the major damaging species produced by activated neutrophils. While HClO itself is a destructive oxidant, it also may interact with low molecular mass iron or O2 •) to produce the •OH. Nitrotyrosine concentrations also are significantly elevated in the BAL fluid protein of patients with RDS [Ogihara, T. et al. (1999)]. Nitration of tyrosine residues is an in vivo marker of the formation of ONOO). Earlier studies reported increased nitrotyrosine concentrations in the lungs of patients with RDS. Additionally, under acidic conditions ONOO) decomposes to form a powerful oxidant with properties similar to •OH. There is, however, another possible explanation for the formation of nitrotyrosine. Recent work shows that nitrotyrosine can arise from the reaction of tyrosine with nitroxyl chloride, an intermediate formed by the interaction of nitrite (the auto-oxidation product of nitric oxide) with HClO. Interestingly, nitrotyrosine concentrations in BAL fluid protein from patients with RDS treated with NO were elevated compared with those found in lung-injured patients not receiving this therapy [Metnitz, PGH. et al. (1999)]. Increased nitrotyrosine concentrations may reflect augmented ONOO) formation in patients receiving NO. As the patients receiving inhaled NO are no sicker, in terms of Acute Physiology and Chronic Health Evaluation II score or FIO2 requirements, than those patients not receiving this therapy, it implies that inhaled NO may react with O2 •) in these circumstances to form the nitrating agent [Vento, G. et al. (2000)]. Finally, MPO concentrations are significantly elevated in the BAL fluid from patients with RDS, suggesting lung neutrophil recruitment and activation [Yitig, S. et al. (1998)]. Collectively, the data are compelling that RDS is associated with elevated ROS/RNS generation and the consequential increased oxidative damage to the respiratory tree.

Sepsis represents a serious problem in newborns with an incidence of one to 10 cases per 1000 live births, with even higher rates in low-birth-weight neonates. Hospital acquired infections in neonatal intensive care units may also occur as frequently as 30 infections per 100 patients. Mortality rates resulting from sepsis in newborns are 30– 50% [Perez, EM. & Weisman, LE. (1997)]. Sepsis is characterized by alterations in body temperature, hypotension, hypoperfusion with cellular damage which culminates in multiple organ failure [Antonielli, M. (1999)]. The initiating event in sepsis is the result of release of endotoxins [i.e. bacterial cell wall lipopolysaccharides (LPS)] from gram-negative and gram-positive pathogenic bacteria [Lush, CW. & Kvietys, PR. (2000)]. LPS triggers activation of

inflammatory cells, including polymorphonuclear leukocytes (neutrophils; PMN), monocytes/macrophages and lymphocytes. LPS also initiates cellular and humoral aspects of the inflammatory immune response. The inflammatory response that occurs as the result of infection is the predominant determinant of outcome in sepsis [Cohen, J. (2002), Abraham, E. & Singer, M. (2007)]. A major feature of sepsis is tissue infiltration by phagocytic cells [Basit, A. et al. (2006), Cepinskas, G. et al. (2003), Ley, K. & Reutershan, J. (2006), Rawlingson, A. (2003), Razavi, HM. et al. (2005)]. When this occurs, PMN and monocytes/macrophages respond to septic stimulation by producing ROS and RNS [Mochida, S. et al. (2007)]. In addition, PMN release enzymes (e.g. elastase, cathepsin, etc.) and the MPO-derived oxidant, HOCl. These reactants contribute to PMN/macrophage-mediated killing of bacteria. However, if produced in excess during sepsis, the ROS/RNS and proteolytic enzymes cause microvascular dysfunction followed by organ shutdown. An inflammatory response to septic stimuli is crucial for host defense, because it up-regulates anti-inflammatory mediators (e.g. IL-1 receptor antagonist, IL-4, IL-10) and antioxidant enzymes (e.g. CAT, GPx and SOD). However, the excessive production of pro-inflammatory mediators in sepsis overwhelms the anti-inflammatory signaling processes leading to a suppression of innate immune functions (especially those of PMN) and causing immunoparalysis and subsequently an increased susceptibility to infection [Riedemann, NC. et al. (2003)]. It is important to note that, besides immune cells, microvascular endothelial cells also become activated in sepsis which contributes to amplification of the inflammatory response [Lush, CW. & Kvietys, PR. (2000), Ley, K. & Reutershan, J. (2006), Liu, L. & Kubes, P. (2003)]. It is known that septic stimuli (e.g. LPS, TNF-a) initiate activation of transcription factors including NFjB and AP-1, resulting in transcriptional activation of multiple genes. This leads to the release of pro-inflammatory cytokines (e.g. TNF-a, IL-1b, etc), and elevates the expression of adhesion molecules (e.g. E-selectin, ICAM-1, VCAM-1) and chemokines by endothelial cells [Ley, K. & Reutershan, J. (2006), Liu, SF. & Malik, AB. (2006), Abraham, E. (2003), Rao, RM. et al. (2007)]. The central role of ROS/RNS in modulation of the endothelial cell proinflammatory phenotype is well documented [Janssen-Heininger, YM. et al. (2000)]. Despite a large amount of research, little progress has been made in improving the outcome of septic newborns [Riedemann, NC. et al. (2003)]. Efforts to block one or more aspects of the sepsis-associated inflammatory pathways have had little impact on patient survival. Of many drugs tested, few have demonstrated efficacy [Panacek, EA. et al. (2004), Bernard, GR. et al. (2001), Abraham, E. (2005), Ely, EW. et al. (2002)]. Clinical reports are consistent with the involvement of ROS/RNS in neonatal sepsis and its complications. Batra et al. [Batra, S. et al. (2000)] documented increased production of oxygenderived reactants in septic neonates. Also, Seema et al. [Seema, KR. et al. (1999)] found newborns with sepsis have significantly higher levels of TNF-a and increased activity of antioxidative enzymes, SOD and GPx. Finally, Kurt et al. [Nese Citak, KA. et al. (2007)] demonstrated that serum IL-1b, IL 6, IL-8, and TNF-a are mediators of inflammation and can be used at the diagnosis and at the evaluation of the therapeutic efficiency of drugs used to treat neonatal sepsis.

2.2 Oxidative stress and resuscitation with ambient air VS pure oxygen

The traditional method of resuscitation of newly born infants is with pure oxygen [Kattwinkel, J. et al. (1999), Niermeyer, S. et al. (2000)]. However, this therapy was

introduced without any preceding randomised trials being conducted. It was assumed, without any supporting data, that 100% O2 would be the optimal oxygen concentration [Lefkowitz, W. (2002)]. There is, however, reason to believe that oxidative stress is elevated when resuscitation is performed with pure oxygen compared with ambient air. For this reason, SAUGSTAD and AASEN [Saugstad, OD. & Aansen, AO. (1980)] warned that the use of high concentrations of supplemental O2 might be detrimental for resuscitation. Several experimental as well as clinical studies seem to confirm this [Armstead, WM. et al. (1988), Bagenholm, R. et al. (1998), Kondo, M. et al. (2000), Kutzsche, S. et al. (2002), Vento, M. et al. (2001), Saugstad, OD. (2001)]. In addition to animal studies, clinical trials have importantly shown that ambient air is at least as efficient as pure O2 for resuscitation of the newly born [Ramji, A. et al. (1993), Saugstad, OD. et al. (1998), Vento, M. et al. (2001, 2003), Ramji, S. et al. (2003)].

In 1993, RAMJI et al. [Ramji, A. et al. (1993)] published a single-centre study from New Dehli with the aim of investigating the feasibility of using 21% O2 for resuscitation. A total of 42 infants resuscitated with 21% and 42 with 100% O2 were enrolled with the following inclusion criteria: cardiac frequency, 80 bpm and or apnoea/ poor response. Birth weight ,1,000 g and or lethal congenital anomalies were exclusion criteria. Restoration of cardiac frequency, Apgar score, acid base and blood gases were not different between the two groups. A second investigation in this area was the Resair 2 study [Saugstad, OD. et al. (1998)], a multicentre study comprising ,600 infants from Egypt, Estonia, India, Norway, Philippines and Spain recruited from 10 centres. The study was pseudo-randomised and not blinded. No significant differences in the primary outcome measure, which was early neonatal death and/or hypoxic ischaemic encephalopathy grade 2 or 3, were found. However, there was a statistically insignificant tendency to higher neonatal survival in the infants resuscitated with 21% versus 100% O2. Time to first breath and first cry was significantly delayed in those reoxygenated with pure oxygen. Since then, three more studies by Resair 2 collaborators have been published, two from Spain [Vento, M. et al. (2001, 2003)] and one from India [Ramji, S. et al. (2003)]. When the results of these five studies were combined, a significant reduction in neonatal mortality (from 13 to 8%) in those resuscitated with ambient air compared with 100% O2 was found. Most of the 1,737 children were enrolled from developing countries; SAUGSTAD et al. [Saugstad, OD. et al. (2005)], therefore, separately analysed the Spanish babies. In this material, a 3% reduced mortality was found in favour of room-air infants (from 3.5 to 0.5%), indicating that, also in industrialised countries, a significant reduction in neonatal mortality can be achieved by not using pure oxygen for resuscitation. One surprising finding of these studies is that time to first breath and first cry is significantly reduced by 24 s; moreover, the 5-min Apgar score, as well as cardiac frequency at 90 bpm is also significantly higher in room-air-resuscitated infants compared with those resuscitated with pure oxygen.

For babies born at term, the Guidelines of 2005 recommend use of 100% supplemental O2 when a baby is cyanotic or when positive pressure ventilation (PPV) is required during neonatal resuscitation. However, research suggests that resuscitation with ,100% may be just as successful. If resuscitation is started with ,100% oxygen, supplemental oxygen up to 100% should be administered if there is no appreciable improvement within 90 s following birth. If supplemental oxygen is unavailable, the use of room air to deliver PPV is suggested. To reduce excessive tissue oxygenation in a very pre-term baby (less than ,32 weeks), use of an oxygen blender and pulse oximeter during resuscitation is recommended; in this case, begin

PPV with an oxygen concentration between room air and 100%. No studies justify starting at any particular concentration. Adjust O2 concentration up or down to achieve an oxyhaemoglobin concentration that gradually increases toward 90%, and reduce the oxygen concentration as saturations rise over 95%. If the cardiac frequency does not respond by increasing rapidly to .100 bpm, correct any ventilation problem and use 100% oxygen. If the facility does not have use of an oxygen blender and pulse oximeter in the delivery room, and there is insufficient time to transfer the mother to another facility, the resources and oxygen management described for a term baby are appropriate. There is no convincing evidence that a brief period of 100% oxygen during resuscitation is detrimental to the pre-term infant. In 2007, a summary of the results of three systematic reviews [Saugstad, OD. et al. (2005), Tan, A. et al. (2005), Raby, Y. et al. (2007)] of five trials and seven individual studies, including up to 2,011 newborn infants, indicated that neonatal mortality was reduced by 30–40% if resuscitation is carried out with 21% instead of 100% O2 [Saugstad, OD. (2007)]. Room-air resuscitation also leads to faster early recovery and a shorter duration of resuscitation. To date, there are sufficient data available to recommend that newborn resuscitation should generally not be carried out using 100% O2. In extremely low-birth-weight (ELBW) infants, arterial oxygen saturation (Sa,O2) levels should be kept between 85 and 93% or possibly between 88 and 95%, but should definitely not exceed 95%. Fluctuations should be avoided [Saugstad, OD. (2007)]. A recent prospective, randomised, clinical trial included infants of *f*28 weeks of gestation who required active resuscitation and were randomly assigned to a low-oxygen group (fraction of inspired oxygen: 30%) or a high-oxygen group (fraction of inspired oxygen: 90%) [Escrig, R. et al. (2008)]. The fraction of inspired oxygen in the low-oxygen group was increased stepwise to 45% and that in the high-oxygen group was reduced to 45% to reach a stable pulse oxygen saturation of approximately 85% at 5–7 mins in both groups. No differences in oxygen saturation in minute-to-minute registers were found, independent of the initial fraction of inspired oxygen used 4 mins after cord clamping. Likewise, no differences in mortality rates in the early neonatal period were detected. The authors concluded that resuscitation can be safely initiated for ELBW neonates with a low fraction of inspired oxygen (30%), which then should be adjusted to the infant's needs, reducing the oxygen load to the neonate [Saugstad, OD. (2003)].

New Guidelines for Neonatal Resuscitation give reason to Saugstad, who had long been considered a historical mistake and an anachronism to continue to resuscitate infants with 100% O_2. For term infants it's recommended to use oxygen blenders and pulse oximeters during resuscitation; it's recommended the use of O_2 100% only when a child is cyanotic or when is required positive pressure ventilation, but it can be successful resuscitation with concentrations of O_2 <100%; it's recommended to start with 21% oxygen; if there is not an adequate response regarding heart rate within 90 s, add oxygen according to pulse oximetry if possible [Vento, M. & Saugstad, OD. (2010)]. For preterm infants (<32 wk.) it's recommended to begin PPV with O_2 concentrations in a range between 21% and 30%. If Sa O_2 is < 70% at 5 min of age, give oxygen. If heart rate is not increasing satisfactorily, oxygen should be given at any time to both groups. Babies with abnormal lungs, for instance after meconium aspiration, may need supplemental oxygen from early on [Vento, M. & Saugstad, OD. (2010)].

2.3 Oxidative stress and lung injury

Chronic lung disease (CLD) of the newborn is one of the definitive factors influencing the mortality and morbidity of VLBW infants [Banks, BA. et al. (1998)]. The aetiology of CLD is

unknown, but many investigators have suggested that free radicals may have a key role in its development. The exposure of immature lungs to prolonged periods of high levels of inspired oxygen is accepted as an important contributor to the development of CLD through both free radical effects on endothelial and epithelial cell barriers that induce pulmonary oedema and trigger mechanisms that lead to activation and accumulation of inflammatory cells [Saugstad, OD. (1998)]. Unfortunately, CLD still develops in extremely premature infants that do not have significant ventilatory or supplemental oxygen needs in the acute course of prematurity. A new form of CLD is less fibrotic than its earlier counterpart, and there is a significant component of delayed alveolar development and perhaps permanent alveolar underdevelopment [Coalson, JJ. et al. (1999), Margraf, LR. et al. (1991)]. Currently, the mechanisms for the development of the new form of CLD have not been fully elucidated and the contribution of oxygen toxicity is debatable. The fact that premature infants develop CLD without being exposed to high concentrations of supplemental oxygen raises the question as to whether oxidative stress in fact contributes to the development of CLD. It is plausible, however, that even low concentrations of supplemental oxygen in premature patients with developmentally poorly prepared antioxidant defense mechanisms may generate significant oxidant stresses and lung injury secondary to oxidation of specific macromolecules [Welty, SE. (2000)]. In addition, inflammatory cell accumulation and activation in the lung may generate oxidants and oxidant stresses that also oxidise macromolecules which leads to CLD [Welty, SE. (2000)]. The literature provides evidence that premature infants who develop CLD have both qualitative and quantitative differences in oxidation of lipids and proteins when compared with infants who do not develop CLD. Such differences in oxidation patterns are the most obvious in the first few days of life [Welty, SE. (2001)]. In a study by OGIHARA et al. [Ogihara, T. et al. (1999)], plasma levels of lipid aldehydes were measured in the first week of life in premature infants. Plasma concentrations of heptanal, 2-nonenal and 4 hydroxynonenal were higher in the first 24 h of life in infants who develop CLD than in those that did not. In another study, elevated exhaled pentane levels were strongly associated with several adverse outcomes in premature infants. In fact, infants who developed CLD had higher exhaled pentane on the first day of life than did patients who did not develop CLD [Nycyk, JA. et al. (1998)]. Protein oxidation was also previously assessed in premature infants and correlated with the development of CLD. There is also an association between higher protein carbonyl contents in tracheal aspirates in the first week of life and the development of CLD [Varsila, E. et al. (1995)]. Moreover, RAMSAY et al. [Ramsay, PL. et al. (2001)] demonstrated that there were no differences in oxygen requirements of tracheal aspirate contents of total 2,4-dinitrophenylhydrazine reactive proteins between premature infants who did or did not develop CLD; however, infants who developed CLD did have more frequent oxidation of specific proteins than did infants who did not develop CLD. These results suggest that identifying specific proteins that are more frequently oxidised in infants who develop CLD may be important in determining specific mechanisms for the development of CLD. Other pathways of ROS generation include metabolism of catecholamines, the arachidonic acid cascade, and mitochondrial metabolism [Bracci, R. (1997)]. However, the main source of free radicals in the lungs seems to be phagocyte activation [Delacourt, C. et al. (1996), Pittet, JF. et al. (1997)]. The increase in phagocyte number and interleukin concentrations in BAL fluid obtained from premature infants with CLD indicates that oxygen toxicity and inflammation

are involved in the development of lung injury [Groneck, P. et al. (1993)]. Infants destined to develop CLD have increased pro-inflammatory cytokine levels in airway samples [Bancalari, E. & Gonzales, A. (2000), Speer, CP. & Groneck, P. (1998)]; however, there is little information on when the pro-inflammatory indicators appear or how they progress in the pre-term lung subjected to mechanical ventilation. Moreover, in those infants, a large number of activated neutrophils are found in the air spaces within hours after birth [Arnoon, S. et al. (1993)].The contribution of airway inflammation to the development of CLD of prematurity has been extensively studied [Jonsson, B. et al. (1997), An, H. et al. (2004), Bancalari, E. (2000), Groneck, P. & Speer, CP. (1995), McColm, JR. & McIntosh, N. (1994)]. There is a dynamic and complex balance between pro- and anti-inflammatory cytokines in the human immune system. Previous studies on premature infants have shown that an increase of tumour necrosis factor (TNF)-a in tracheal secretions, among other pro-inflammatory cytokines, was associated with the duration of mechanical ventilation [Schultz, C. et al. (2003)] and the development of CLD [Deng, H. et al. (2000), Kotecha, S. (1996), Kotecha, S. et al. (1995)]. The role of the anti-inflammatory cytokines is less clear. Recent studies have demonstrated that pre-term infants with respiratory distress do produce significant amounts of IL-10 in the lower airways and the presence of this anti-inflammatory cytokine prevents the development of CLD of prematurity [McColm, JR. et al. (2000)]. JONES et al. [Jones, CA. et al. (1996)] were unable to detect interleukin (IL)-10 in most of the airway samples from pre-term infants. This observation agrees with a study showing that the control of airway inflammation by this cytokine is limited in infants [Dudley, DJ. et al. (1997)]. Of interest is that SAUGSTAD [Saugstad, OD. (2003)] claims that these changes are seen very early and are present only a few hours or days after birth in those infants who go on to develop CLD. This may support the suggestion that pre-natal factors, such as inflammation, are important for its development and that the changes leading to CLD are triggered before birth. If this is the case, it holds important implications for future therapeutic approaches [Saugstad, OD. (2003)]. The most common reason for neonates requiring respiratory support is RDS. In this disease, the pathophysiology is one of progressive loss of lung volume, intrapulmonary shunt and deflation instability. Animal and human models of RDS have clearly shown that ventilator strategies alter the clinical and pathological evolution of RDS. In addition, it is claimed that neonates with RDS are susceptible to lung injury and the subsequent development of related conditions. It is being increasingly realised that modes of mechanical ventilation that result in end-inspiratory alveolar over-stretching and/or repeated alveolar collapse and re-expansion disturb the normal fluid balance across the alveolo-capillary membrane. The effects of this include disturbance in the integrity of the endothelium and epithelium and impairment of the surfactant system; these changes are similar to those seen in acute RDS. Mechanical ventilation can injure pre-term lungs and multiple ventilation strategies have been attempted to reduce injury and improve outcomes. In 1999, CLARK et al. [Clark, RH. et al. (1999)] proclaimed that, ‘‘the concept of ventilator-induced lung injury has come of age’’. There are many data which suggest that ventilation can cause biotrauma associated with a ‘‘mediator storm’’ (perhaps cytokines) and that it is responsible for distal organ dysfunction, subsequent multiorgan failure and death. Although it has been shown that pulmonary cytokine levels also appear to be elevated in some neonates on assisted ventilation, the exact relationship to neonatal lung injury has yet to be defined. Pro-inflammatory mediators may

be elevated because of fetal exposure to maternal inflammatory mediators, post-natal infections or due to release of mediators from the pre-term lung attributable to ventilator-induced injury. The pre-term lung is susceptible to injury with the initiation of ventilation because potential lung volumes are small, surfactant may be deficient, the lung matrix is not fully developed and the air spaces contain residual fetal lung fluid. Tidal volume (VT) during the resuscitation of pre-term infants is not monitored, and easily visible chest movements will result in VT in excess of that routinely needed to ventilate infants [Ikegami, M. et al. (2000)]. Preterm infants are often hyperventilated and low carbon dioxide tension (PCO2) values after birth correlate with an increased incidence of CLD [Gannon, M. et al. (1998)]. The most effective strategy is the avoidance of mechanical ventilation with the use of nasopharyngeal continuous positive airway pressure whenever possible. Barotrauma, volutrauma and oxygen toxicity, during intermittent positive pressure ventilation, are assumed to be important factors in the pathogenesis of CLD as they cause pulmonary damage, resulting in a release of multiple proinflammatory cytokines and production of extracellular matrix components and growth factors [Ehrenkranz, RA. & Mercurio, MR. (1992)]. The current orientation in the clinical practice is to emphasise the potential importance of reducing mechanical insults on acutely diseased lungs by using special modes of ventilation, e.g. high frequency oscillatory ventilation (HFOV), that limit the pressure and volume of gas delivered to the lungs [Zoban, P. & C ˇ erny, M. (2003)]. HFOV may reduce volutrauma by using a small tidal volume (VT), maintaining almost constant alveolar pressure, and optimising lung volume through the regulation of mean airway pressure [Bohn, DJ. et al. (1980), Gerstmann, DR. et al. (1990)]. Reducing volutrauma is important since damaged tissue generates free radicals and may become inflamed, a process that further contributes to the production of toxic oxygen derivatives. The results of randomised trials to date, conducted on human neonates comparing HFOV with conventional mechanical ventilation (CMV), have been inconclusive and the results are conflicting. Therefore, it remains an open question whether HFOV is more beneficial in preventing CLD than a high-rate, minimal-pressure, low VT, CMV strategy [Bollen, CW. et al. (2003)]. Also, in the Cochrane Database of 2007, the authors conclude that there is no clear evidence that elective HFOV offers important advantages over CMV when used as the initial ventilation strategy to treat pre-term infants with acute pulmonary dysfunction. There may be a small reduction in the rate of CLD with HFOV use, but the evidence is weakened by the inconsistency of this effect across trials and the overall borderline significance [Cools, F. et al. (2007)]. To develop less traumatic mechanical ventilation, with the aim of limiting lung volutrauma, guaranteed volume (GV) integrates various modalities to trigger ventilation with pressure control including assisted/controlled (A/C), synchronised intermittent mandatory ventilation (SIMV) and pressure support ventilation (PSV). GV is an uncommon ventilation method which controls pressure but provides a fixed current volume according to compliance variations, to resistance and to spontaneous activity. The ventilator corrects inspiratory pressure giving a current volume that tends to be the same as the set volume. The gradual improvement in compliance of a pulmonary pathology ventilated with GV follows a reduction of peak inspiratory pressure [Donn, SM. & Sinha, SK. (2003), Herrera, CM. et al. (2003)]. LISTA et al. [Lista, G. et al. (2004)] evaluated the lung inflammatory response in pre-term infants with RDS mechanically ventilated with or without GV, by measuring pro-inflammatory cytokines (IL-6, IL-8, and TNF-a) in

tracheobronchial aspirate (TA) fluid. Their data suggest that a volume-targeted ventilatory strategy may play a role in reducing the acute inflammatory response, and thereby also limiting oxidative stress in pre-term infants with RDS. The outcome of this clinical trial shows that there are lower pro inflammatory cytokine levels (IL-6, IL-8, and TNF-a) in BAL of infants with severe RDS supported with PSV with GV compared with PSV only. The study of DANI et al. [Dani, C. et al. (2006)] was the first clinical trial demonstrating that the early treatment of RDS with HFOV is associated with the reduction of pulmonary inflammatory reaction in pre-term infants in comparison with the early application of another potentially lung-protective ventilations strategy such as PSV plus GV. While there are obviously conflicting findings in this field, it is generally accepted that antecedent lung inflammation or injury makes the lungs more susceptible to volutrauma and oxidant induced injury by the reactive species shown in figure 1. The resulting damage promotes inflammation that is not limited to the lung but that may also affect distant organs, and oxygen, when used at high concentration, can be toxic. Although there are a variety of modalities of ventilation that are non invasive, each ventilatory strategy has a potentially negative consequence in terms of tissue damage resulting from the production of both ROS and RNS. Small VT ventilation is associated with progressive low volume and surfactant dysfunction. Limiting VT requires higher levels of end-expiratory pressure and/or FI,O2 to maintain adequate oxygenation. Higher levels of FI,O2 can contribute to oxidant-induced lung injury. Thus, the use of lung protective strategies in the neonate requires proactive decisions that must be specific for disease pathophysiology and lung maturity, and that involve compromises between gas exchange goals and potential toxicities of the treatments. Besides the ventilatory strategies in common use to treat brochopulmonary dysfunction in newborns and because optimal oxygen saturation for use in these cases is difficult to achieve, other treatments have also been attempted. For example, inhalation of nitric oxide, administration of caffeine or surfactant and intramuscular injection of high doses of vitamin A have been used in infants with the hope of improving pulmonary physiology. Additionally, the utility of two antioxidants, i.e. Nacetylcysteine and superoxide dismutase, have been tested. None of these extra-ventilatory procedures has generally provided substantial benefit [Thomas, W. & Speer, CP. (2008)].

3. Melatonin and oxidative stress

Melatonin, an endogenously produced indoleamine, is a highly effective antioxidant, free radical scavenger, and a primary circadian regulator. Melatonin has important antioxidant properties owing to direct and indirect effects. It directly scavenges reactive oxygen and reactive nitrogen species, prevents molecular oxidation, improves mitochondrial physiology, and restores glutathione homeostasis. Its indirect antioxidant effects stem from its ability to stimulate the activities of the enzymes involved in the glutathione cycling and production. Melatonin, by reducing free radical damage, may be an effective protective agent for the fetus as it is in adults. Several clinical studies on melatonin have shown that it reduces oxidative stress in human newborns with sepsis, hypoxic distress, or other conditions, where there is excessive free radical generation. Several clinical studies that used melatonin showed that it reduces oxidative stress in newborns with sepsis, distress, or other conditions, where there is excessive ROS/RNS production [Gitto, E. et al. (2009)]. In one of these studies, the level of lipid peroxidation products and the nitrite + nitrate levels were

measured in the serum of asphyxiated newborns before and after treatment with melatonin given within the first 6 hr of life [Fulia, F. et al. (2001)]. Serum levels of these measures in newborns with asphyxia were found to be significantly higher than those in normal infants; these levels were significantly reduced by melatonin treatment [Fulia, F. et al. (2001)]. The protective actions of melatonin in these situations likely relate to the antioxidant properties of the indole and its metabolites as well as to the ability of melatonin to increase the efficiency of mitochondrial electron transport, thereby reducing electron leakage and free radical generation [Hardeland, R. (2005), Leon, J.; Acun˜a-Castroviejo, D. et al. (2004), Leo´n, J. et al. (2005), Reiter, RJ. et al. (2008)].

A seminal study examined the changes in the clinical status and serum levels of lipid peroxidation products [MDA and 4-hydroxylalkenals (4-HDA)] in septic newborns treated with melatonin given within the first 12 hr after diagnosis [Gitto, E. et al. (2001)]. Ten other septic newborns in a comparable state were used as _septic_ controls, while 10 healthy newborns served as normal controls. Serum MDA + 4-HDA concentrations in newborns with sepsis were significantly higher than those in healthy infants without sepsis; in contrast, in septic newborns treated with melatonin, there was a significant reduction of MDA + 4- HDA levels compared to the values measured in the normal controls at both 1 and 4 hr after the initiation of melatonin treatment. Melatonin also improved the clinical outcome of the septic newborns as judged by measurement of sepsis related serum parameters after 24 and 48 hr. We have also tested whether melatonin treatment would lower IL-6, IL-8, TNFa, and nitrite/nitrate levels in 24 newborns with respiratory distress syndrome (RDS) of III or IV grade (radiographically confirmed) diagnosed within the first 6 hr of life [Gitto, E. et al. (2004)]. Compared with the melatonin treated RDS newborns, in the untreated infants, the concentrations of IL-6, IL-8, and TNFa were significantly higher at 24, 72 hr, and at 7 days after onset of the study. In addition, nitrite/nitrate levels at all time points were higher in the untreated RDS newborns than in the melatonin treated babies. Following melatonin administration, nitrite/ nitrate levels decreased significantly, whereas they remained high and became further elevated in the RDS infants not given melatonin. In a clinical trial, the levels of proinflammatory cytokines (IL6, IL-8, and TNFa) and the clinical status of 110 preterm newborns with RDS ventilated with different modalities [conventional ventilation, pressure-support ventilation (PSV) and with guaranteed volumes (GV), and high-frequency oscillatory ventilation] were evaluated before and after treatment with the antioxidant melatonin [Gitto, E. et al. (2005)]. Compared with the melatonin-treated RDS newborns, the concentrations of inflammatory cytokines were significantly elevated in the newborns given only the diluent. When serum levels of IL-6, IL-8, and TNFa for the two groups were compared, melatonin treatment clearly had anti-inflammatory effects. In particular, it was noted that newborns mechanically ventilated in PSV mode with GV presented a greater reduction of serum levels of inflammatory cytokines than did newborns ventilated in conventional mode or with oscillatory ventilation. The measured inflammatory cytokines were most markedly elevated in infants mechanically ventilated but not given melatonin. Newborns not treated with melatonin, who developed chronic lung disease (CLD), have much higher concentrations of proinflammatory cytokines than infants without CLD [Gitto, E. et al. (2004)]. It was also found that melatonin lowered interleukin IL-6, IL-8, TNF-a, and nitrite/nitrate levels and modified serum inflammatory parameters in surgical neonates, thereby improving their clinical course [Gitto, E.; Romeo, C. et al. (2004)]. In animals, the anti-inflammatory actions are thoroughly described [Rodrı´guez, MI. et al. (2007)].

4. Conclusion

Toxic derivatives of oxygen are referred to as free radicals and are either oxygen (ROS) or nitrogen-based (RNS) reactants. ROS/RNS are destructive to all key molecules, i.e. lipids, proteins and DNA, within all cells. Since the lungs of newborn infants are highly susceptible to oxidative damage by ROS/ RNS, care should be taken in the use of pure oxygen during resuscitation of infants. Also, avoidance of mechanical ventilation with the use of nasopharyngeal continuous positive air pressure may reduce respiratory tissue damage resulting from ROS/RNS.

Oxygen, which is obviously vital to survival, can obviously be highly damaging to tissues such as the lungs of newborns which are known to be poorly equipped to neutralise its toxic derivatives. Thus, the exposure of the newly born infant respiratory tree to oxygen at a higher percentage than exists in normal ambient air, i.e. 20%, or at a positive pressure should be performed with caution especially since it may be minimally or no better than using ambient air. Also, the use of antioxidants to quell molecular damage by ROS/RNS could be considered in situations in which pure oxygen or positive pressure are used. One antioxidant that may be useful in these situations is melatonin; this indoleamine has been shown to be useful to combat oxygen toxic in newborns [Gitto, E. et al. (2009)].

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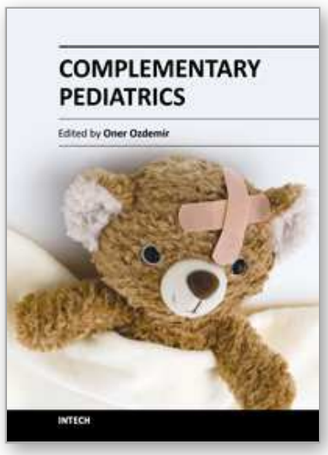

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