Hyperoxia and the Immature Brain

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Abstract

Despite major advances in obstetrics and neonatal intensive care, preterm infants frequently suffer from neurological impairments in later life. Preterm and also full-term neonates are generally susceptible to injury caused by reactive oxygen species due to the immaturity of endogenous radical scavenging systems. It is well known that high oxygen levels experienced during the critical phase of maturation can profoundly influence developmental processes. Supraphysiological oxygen concentrations used for resuscitation or in the care of critically ill infants are known to have deleterious effects on the developing lung and retina, contributing to the pathophysiology of neonatal diseases like bronchopulmonary dysplasia and retinopathy of prematurity. Moreover, experimental work from the last decade suggests that hyperoxia also leads to neuronal and glial cell death, contributing to the injury of white and grey matter observed in preterm infants. During the critical phase of brain maturation, hyperoxia can alter developmental processes, resulting in the disruption of neural plasticity and myelination. However, oxygen therapy can often not be avoided in neonatal inten-

sive care. Therefore, in situations requiring oxygen supplementation, in addition to the development of appropriate monitoring systems, protective and/or regenerative strategies are highly warranted. Here, we summarise the clinical and experimental evidence as well as potential therapeutic strategies, providing an overview of the pathophysiology of oxygen exposure on the developing central nervous system and its impact on neonatal brain injury.

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“Oxygen? I rarely use it myself, sir. It promotes rust.”
Robby the Robot, *Forbidden Planet* (1956)

Introduction

Major advances in obstetrics and neonatal intensive care have substantially improved survival of very preterm and critically ill full-term infants [1]. Perinatal mortality has decreased by 25% over the last decade and has expanded the surviving population. However, despite all efforts, perinatal brain injury is still a leading cause of death and disability in children [2]. Premature birth can affect brain development at an early age with life-long individual, familial, and socioeconomic consequences [3].

The percentage of prematurely born infants has increased in industrialised countries during the last decades, and accounts for 5.5–11.4% of all live births [4, 5]. This number is likely to rise further due to the increasing rate of infertility treatments, multiple pregnancies, and older mothers [6]. Intact long-term survival for premature infants has become an almost expected outcome over the past two decades. Improvements in neonatal respiratory care have expanded the very low birth weight (VLBW) population (a birth weight <1,500 g) accounting for approximately 2.5% of total annual births [7]. With an improvement in the survival rate, the focus of clinical efforts has shifted to the immediate and later consequences of prematurity. Unfortunately, a substantial proportion of patients still have neurologic deficits, which affect motor and cognitive function [8–10]. Approximately 10% of survivors of very preterm birth suffer from periventricular leukomalacia, and later exhibit motor deficits characteristic of cerebral palsy [11]. Even in the absence of obvious intracranial pathology such as intraventricular haemorrhage or periventricular leukomalacia, preterm infants are at a high risk for neurodevelopmental impairment. Due to considerable progress in the perinatal management of high-risk infants, major focal destructive lesions have become less common. Diffuse white matter injury (WMI) and a reduction of cortical grey matter volume are observed in most survivors, and are often associated with cognitive impairment, attention deficit disorder, behavioural problems, autism, and development of psychiatric disease in later life [12–15].

Developmental Brain Injury

The development of the mammalian brain is a dynamic process involving structural and functional maturation processes. Brain evolution is characterised by neuronal cell development and proliferation, migration, glial cell proliferation, axonal and dendritic outgrowth, synaptogenesis and the myelination of axons [16]. At the border of viability of extremely preterm infants (around 24 weeks of gestation), neuronal migration processes are generally completed, but glial cell maturation, outgrowth, and the formation of connectivity are still in progress [17, 18]. The formation of neural electric activity strongly depends on metabolic factors such as mitochondrial development, cerebral vascular density and blood flow, the maturation of glucose utilisation systems, and cytochrome oxidase activity [19, 20].

During physiological brain development, initially formed supernumerary neurons are deleted by physiological apoptosis. During a perinatal insult, accidental activation of the well-refined apoptotic cell death machinery may occur [21]. Apoptosis is executed via 2 different signalling routes. The extrinsic pathway involves extracellular signalling via cell death surface receptors (i.e., Fas and TNF- α) and the intrinsic pathway is activated by cellular stress or DNA damage [22]. Both pathways can converge downstream at the mitochondrial level. Upon a strong injurious trigger, the mitochondrial inner-membrane potential decreases and induces permeability with the release of pro-apoptotic factors (i.e., apoptosis-inducing factor [AIF] and cytochrome c) in the cytosol, which activates death mechanisms including the formation of the active apoptosome (apoptosis protease-activating factor-1 [Apaf-1]) [22]. These mechanisms are modulated by several intrinsic factors such as the members of the Bcl-2 family. It is well known that free radicals, formed during altered oxygen tension, can lead to direct DNA damage as well as the leakage of mitochondrial membranes with the release of cytochrome c into the cytoplasm, and the activation of caspases [23–25]. Depending on the type of insult, caspase-dependent and caspase-independent apoptotic signalling are induced [26, 27]. The majority of apoptotic factors including caspases are highly expressed in the developing brain, resulting in the increased susceptibility of the immature organism to injurious activation. Moreover, caspases seem to have important non-apoptotic functions in multiple cellular processes, such as synaptic plasticity, dendritic development, learning, and memory [28].

Taking immaturity and potentially damaging factors into account, the pathophysiology of perinatal brain injury is complex and involves grey and white matter structures in varying degrees, depending on gestational age, developmental stage, and injury stimulus [29]. In preterm infants, the reduction of brain volume, cortical folding, and delayed maturation associated with adverse neurological development are common findings on magnetic resonance imaging (MRI) [30]. Two major causes are generally considered to be responsible for this specific encephalopathy of prematurity: cerebral ischemia/reperfusion combined with the propensity for impaired vascular autoregulation and infection in the mother and/or foetus that triggers a cytokine response in the foetal brain [31–34]. Moreover, several perinatal factors such as growth factor deficiency, drug exposure, maternal stress, malnutrition, and also genetic factors have been studied, and are likely to be important players in the

Fig. 1. Perinatal increase of the oxygen tissue tension and detrimental effects of oxygen on the developing infant.

pathophysiology of brain lesions associated with worse neurological outcome [review: 35, 36]. Furthermore, the possible long-term effects of glucocorticoids, given to women threatening preterm labour, and the consequences of the postnatal application of steroids have still to be determined in more detail [37, 38]. Since there has been no improvement in the neurodevelopmental outcome of premature infants in the past decade [39], the search for other factors that might be involved in perinatal brain injury as well as for potential protective strategies is ongoing.

It has been known for a long time that treating premature infants with high oxygen levels results in retinopathy of prematurity (ROP), leading to severe visual impairment and blindness [40, 41]. However, the induction of mechanical ventilation with high concentrations of oxygen improves the survival of critically ill neonates with respiratory distress. Meanwhile, oxygen has also been identified as one of the principal factors in the pathogenesis of chronic lung disease of prematurity due to its detrimental effects on lung development [42]. There is mounting evidence from various clinical and experimental observations to suggest that hyperoxia, among other sources of oxidative stress, is an important trigger of brain injury [43–48]. Chronic exposure to supraphysiological oxygen concentrations may lead to the malformation of neuronal circuits during development, with a dramatic deterioration of brain function in later life [49]. Foetal development occurs under relative hypoxic conditions in utero (a PaO_2 of approx. 25 mm Hg) compared to extrauterine conditions (a

PaO_2 of 70 mm Hg) (Fig. 1). The switch from placenta- to lung-mediated oxygen supply during birth is associated with a sudden rise of tissue oxygen tension that amounts to relative hyperoxia in preterm infants, and supplemental oxygen application intensifies the pathophysiological situation [50]. Hyperoxia, during development in rats, results in hypoxic chemosensitivity ablation, carotid body hypoplasia, and reduced chemoafferents [51, 52]. Moreover, normoxia after hypoxia (8% O_2) induces oxidative stress by increasing reactive oxygen species (ROS) levels by 19.2%, with a further increase of 54.8% with hyperoxia (95% O_2) compared to normoxia [53].

In summary, defining the optimum oxygen application in preterm infants and developing safety monitoring systems are important goals for the future. Since oxygen is an important component of neonatal resuscitation and treatment, gaining knowledge about its possible side effects and developing promising adjuvant therapy options are very important for future clinical and experimental investigations and neonatal care.

Oxygen Vulnerability and Developmental Brain Injury

Fluctuating environmental oxygen conditions play a substantial role in cellular and organismal respiration and evolution. A large number of studies have confirmed the role of hyperoxia in the pathogenesis of prematurity-related diseases such as ROP and bronchopulmonary dys-

plasia (BPD) [42, 54]. The clinical and experimental insights of the past decade have investigated how premature exposure to large amounts of oxygen in the neonatal period may disrupt brain maturation. There is mounting evidence that hyperoxia has deleterious effects on the immature brain. In preterm infants, chronic or fluctuating exposure to supraphysiological oxygen levels compared to intrauterine conditions may cause encephalopathy of prematurity with cystic or diffuse periventricular leukomalacia [55, 56]. In full-term infants suffering from birth asphyxia resuscitation with high oxygen concentrations significantly increases mortality and morbidity [57]. In rodents, hyperoxia-triggered subtle neurodegeneration is associated with apoptosis, autophagy, changes in the expression of neurotrophins and growth factors, oxidative stress, inflammation, and alterations in genes related to synaptic plasticity. Furthermore, transient hypomyelination may lead to long-lasting microstructural changes in the white matter [48, 58–62]. Recent studies on rodents further revealed hyperactivity and coordination deficits in adolescence and cognitive impairment persisting into adulthood, i.e., conditions that preterm infants also suffer from in later life [60, 63, 64].

Experimental Models

Intracellular Effects of Hyperoxia

Apoptosis and Autophagy

In humans, the period of rapid brain growth, i.e., the period of highest vulnerability to injurious stimuli, is observed during the last 3 months of pregnancy. In contrast, newborn rodents have their growth spurt between postnatal day 2 (P2) and P10 [65–67]. Such species are therefore used as experimental models to investigate the mechanisms of vulnerability in the developing brain. In 2004, a preterm animal model of hyperoxia-induced developmental brain injury was established, where 6-day-old rodents were subjected to increased oxygen levels (80%) over a period of 2–48 h. Under these conditions, hyperoxia causes a widespread pattern of increased neuronal apoptosis, when compared to the physiological apoptosis typically seen at this developmental stage. The areas affected are the cortex, basal ganglia, hypothalamus, striatum, hippocampus, and white matter tracts [47, 68]. Interestingly, oxygen sensitivity is maturation-dependent, as 14-day-old rats are resistant to the effects of a rapidly increasing oxygen supply; upon analysis, only the dentate gyrus of the hippocampus revealed areas of increased apoptotic cell death (Fig. 2) [47]. The

treatment of immature rats with high doses of oxygen from birth in the first 5 days of life also results in a significant increase in apoptotic cells and a reduction of brain weight [69]. Analysis of apoptotic pathways after 2–72 h of 80% oxygen exposure in 6-day-old rodents revealed receptor-mediated apoptosis, as hyperoxia resulted in the induction of Fas and its downstream signalling events, such as Fas-associated death-domain (FADD), the long and short form of FADD-like-IL-1 β -converting enzyme (FLICE)-inhibitory protein (FLIP-L and FLIP-S), and the cleavage of caspase-8 and caspase-3. Mice deficient in Fas (B6.MRL-Tnfrsf6^{lpr}) were protected against oxygen-mediated injury [59]. In the same injury model, hyperoxia initiates intrinsic apoptotic pathways with the upregulation of key proteins, namely, cytochrome c, Apaf-1, and the caspase-independent protein AIF, but members of the antiapoptotic Bcl-2 family are downregulated [59, 69–72]. These results coincide with an upregulation of caspase-3 activity and marked neurodegeneration [71].

Recently, autophagy has also been described as a prominent feature of cell death during brain development, and cross-talk between autophagy and apoptosis has been described. Autophagy seems to be protective in the early stages of programmed cell death, but it can also promote apoptosis under particular circumstances such as hypoxia-ischemia [73, 74]. Proteins well-described in this process are Beclin-1 and microtubule-associated protein 1 light chain 3 (LC3) [75, 76]. For the developing brain, data on autophagic pathways is sparse [74, 77]. Still, autophagy as a self-degradation process that involves the turnover and recycling of cytoplasmic components in healthy and diseased tissue seems to be involved in hyperoxia-induced developmental brain injury. A time-dependent upregulation of the autophagy-related gene (*Atg*) proteins Atg3, 5, 12, Beclin-1, LC3, LC3A-II, and LC3B-II was observed in 6- to 7-day-old rats after 12 h of hyperoxia, and these were downregulated at 24 h, respectively [78]. Both apoptosis and autophagy seem to contribute to the pathogenesis of hyperoxia-induced brain damage.

Neurotrophins and Neurotransmitters

Neurotrophins are a family of proteins involved in the growth, differentiation, and development of the nervous system. They provide trophic support to the developing cells of the central nervous system (CNS), and their withdrawal may lead to neuronal death [79, 80]. Two well-known, widely distributed neurotrophins in the CNS are brain-derived nerve growth factor (BDNF) and glial-de-

Fig. 2. Distribution pattern, time, and age dependency of hyperoxia-induced apoptosis in infant rats. Figure was taken from Felderhoff-Mueser et al. [47]. **a** Schematic illustration of the distribution of apoptotic cells in brains of P7 rats subjected to 24 h of hyperoxia. Dotted areas are affected. **b** Numerical density of degenerating cells in 11 brain regions in normoxic rats and in rats subjected to hyperoxia for 12 or 48 h ($n = 8$ in each group). This was significantly increased in all brain regions after both periods of hyperoxia compared to normoxic rats. Fr, frontal cortex (layer II and IV); Par, parietal cortex (layer II and IV); Cing, cingulum (layer II and IV); Caud, nucleus caudatus; WM, white matter; LD thal, laterodorsal thalamus; Sub, subiculum; N. ac, nucleus accumbens. **c** Impact of duration of hyperoxia exposure on the severity of

apoptotic cell death: P7 rats were exposed to 80% O_2 for 2–72 h and sacrificed at either 24 h (applies to exposure periods of 2–24 h) or at the end of the exposure (applies to exposures of 48 and 72 h; $n = 7–10$ /time point). A 2-h exposure to an 80% O_2 environment was sufficient to trigger significant apoptotic cell death compared to normoxic litter mates. There was severe degeneration in the brains of rats subjected to 12 or 24 h of hyperoxia. No further increase was detected after longer exposure times, most likely because apoptotic cells had already been eliminated. **d** Developmental vulnerability profile to hyperoxia: P0–P14 rats ($n = 7–10$ /group) were exposed to 80% O_2 for 24 h and were sacrificed at the end of the exposure. Vulnerability to hyperoxia subsided by P14. ** $p < 0.01$; *** $p < 0.001$; Student t test: hyperoxia vs. normoxia.

rived nerve growth factor (GDNF). Their expression during early brain development contributes to neuronal migration, survival, and maintenance [81–83].

In the developing brain of 6-day-old rats after hyperoxia exposure, cell death has been shown to be associated with reduced expression of neurotrophins and neurotrophin-regulated signalling pathways, including that of extracellular signal-regulated kinase (ERK) [47, 59, 69, 72]. In mice subjected to hyperoxic conditions from P7

to P12, the expression of BDNF and GDNF is decreased, potentially leading to alterations in neural development [84]. In a newborn piglet model of global hypoxia/reoxygenation, levels of the neurotrophic factor BDNF are reduced when the animals receive 100% oxygen for resuscitation [85, 86]. In summary, it appears that a decrease in neurotrophins is induced by hyperoxia treatment, possibly leading to a reduction of intrinsic neuroprotective properties.

ROS and ROS-Dependent Systems

ROS, such as hydroxyl radicals, hydrogen peroxide, superoxide anions, or singlet oxygen, are chemical species containing oxygen, and they occur as a physiological reaction to an oxygenated environment during oxygen metabolism. Physiologically, ROS are counterbalanced by the antioxidant defence system [87]. A disturbed balance between ROS production and antioxidant defence can cause oxidative stress, which is associated with damage to the DNA and mitochondrial membrane, apoptosis, and cellular dysfunction [23–25, 88]. Peroxidation of proteins, lipids, and polysaccharides may occur in this context. The immature brain is particularly vulnerable because of its high oxygen intake and the high content of polyunsaturated fatty acids [89]. Experiments on rodents revealed excessive amounts of ROS generation upon stimulation in comparison to the adult brain [90]. High numbers of mitochondria make synapses highly susceptible to oxidative stress, which may either lead to synapse loss, as a result of impaired mitochondrial function, or to synaptic overgrowth [91, 92]. Since there is great commonality between the pathways involved in synapse function and development and those who contribute to oxidative stress, ROS may also play a role in synaptic signaling during cognitive processes such as learning and memory [93].

The CNS has a high rate of oxygen consumption, which can result in an excess production of ROS, especially under hyperoxic conditions [94–96]. Moreover, hyperoxia can increase the production of ROS in CNS cells, resulting in enhanced cell death *in vivo* and *in vitro* [48, 53, 95, 97].

The mammalian organism has evolved inducible responses to ROS that are generated as a consequence of physiological metabolism. The most studied antioxidant enzymes in the developing brain are manganese-containing superoxide-dismutase (Mn-SOD), copper- and zinc-containing SOD (CuZn-SOD), glutathione-peroxidase (GPx), glutathione, vitamins A, C, and E, and catalase. Mitochondria can produce SOD, GPx, and glutathione reductase. The immature mammalian brain physiologically needs these antioxidant enzymes to protect against oxidative stress that occurs at birth due to the relative hyperoxia compared to intrauterine conditions. The expression of SOD, catalase, and GPx increases by 150% in the third trimester of pregnancy [98]. Furthermore, the development of antioxidant capacities during the foetal period is associated with redox signalling for the maintenance of pregnancy [98, 99]. Local nitric oxide generation as a relatively weak oxygen free radical in the placenta is important for vascular development. Mn-SOD seems

crucial for the protection of immature oligodendrocytes (OLs), and the production of CuZn-SOD increases significantly during myelination in postnatal rat brains [98, 100]. The influence of oxidative stress in the developing white matter has also been well investigated; Baud and coworkers [44, 45, 101] demonstrated that nitric oxide is more toxic to developing OLs than to mature OLs. Maturation-dependent vulnerability of premyelinating OLs (pre-OLs) to oxidative stress has been confirmed in several paradigms. Thus, oxidative stress produced by glutathione depletion results in marked cell death in pre-OLs, while mature OLs are resistant. This vulnerability appears to be related to the accumulation of free radicals in pre-OLs but not in mature cells, suggesting a defect in the ability of immature OLs to remove ROS. This inability to remove ROS is related to relative deficiencies of antioxidant enzymes [44, 45, 101].

It has been shown that hyperoxia triggers an increase in oxidative stress by modulating intracellular redox homeostasis, via an increase in oxidised glutathione and a decrease in reduced glutathione and heme oxygenase 1 (HO-1) as well as an increase in lipid peroxidation in the immature brain of 6-day-old rats after 2–48 h of hyperoxia [72, 102]. In this model, even short exposures to non-physiologic oxygen levels can change the balance of the ROS-dependent thioredoxin/peroxiredoxin system. Oxygen toxicity significantly induces the upregulation of peroxiredoxins 1 and 2, peroxiredoxin sulfonic form, thioredoxin 1, and sulfiredoxin 1 in the brains of immature rats. Moreover, hyperoxia reduces the level of DJ-1, a hydroperoxide-responsive protein in the developing rat brain [103]. Hyperoxia exposure also leads to oxidative, nitrosative, and nitrative stress, ensuing microvascular degeneration, diminished brain mass, and neurophysiological function in immature rat pups [104]. These effects are preceded by an upregulation of endothelial nitric oxide synthase (eNOS) in the cerebral capillaries and a downregulation of Cu/Zn-SOD. A role for reactive nitrogen species in hyperoxic death is suggested by the observation that hyperoxia causes the upregulation of inducible (i)NOS mRNA and protein in microglial cells, and the formation of nitrotyrosine in the neurons of the immature rat brain [96]. Therefore, hyperoxic brain injury is accompanied by high levels of oxidative stress with the formation of ROS as well as a reduction of antioxidant defence mechanisms.

Inflammation

Intrauterine infection is a major cause of preterm birth [105, 106]. In the past, experimental and clinical investi-

gations have shown that both inflammation and hyperoxia contribute to preterm brain injury [46, 55, 70, 107–110]. Hyperoxia also generates inflammatory responses in the developing brain, as demonstrated by a marked increase in mRNA and protein levels of caspase-1 and its downstream effectors IL-1 β , IL-18, and IL-18 receptor α (IL-18R α) in 6-day-old rodents exposed to hyperoxia for 2–48 h, whereas intraperitoneal injection of recombinant human IL-18-binding protein (IL-18BP), a specific inhibitor of IL-18, attenuated hyperoxic brain injury [68]. Mice that are deficient in IL-1 receptor-associated kinase 4 (IRAK-4), which is pivotal in both IL-1 β and IL-18 signal transduction, are protected against oxygen-mediated neurotoxicity [68]. These findings causally link inflammation triggered by pro-inflammatory cytokines such as IL-1 β and IL-18 to hyperoxia-induced cell death in the immature brain.

Recently, the hyperoxia model has been modified by the addition of an inflammatory stimulus, which, as already mentioned above, represents a clinically relevant problem in preterm brain injury. To explore the additive or synergistic effects of a combination of treatment with oxygen and inflammation, the influence of a systemic lipopolysaccharide (LPS) application on hyperoxia-induced WMI in newborn rats has been studied [58]. Injection with LPS as an inflammatory stimulus aggravated hyperoxia-induced damage due to microglial activation. The two noxious stimuli, i.e., 24 h of hyperoxia at P6 and LPS, caused hypomyelination and altered the WM microstructure on diffusion tensor MRI. While hyperoxia predominantly induced cell death, LPS also induced OL maturation arrest. The reduced expression of transcription factors controlling OL development and maturation further indicated OL maturation arrest.

Analysis of molecular changes for the disruption of myelination revealed altered expression of the founder molecule, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), in the combined model of hyperoxia- and inflammation-induced encephalopathy of prematurity. Furthermore, primary OLs stimulated with CEACAM1 showed increased myelination [111]. CEACAM1 is part of the immunoglobulin superfamily and is ontogenetically expressed in myelinating OLs. Due to its role as a coreceptor to a variety of other receptors (e.g., Toll-like receptor [TLR]2, TLR4, T cell receptor, B cell receptor, EGFR, and VEGFR) and its different isoforms, CEACAM1 is a multifunctional protein with an impact on proliferation and differentiation [111]. Its effect on other cell types in the context of hyperoxic brain injury remains to be determined.

Effects of Hyperoxia on the Developing White Matter

Recent findings from clinical MRI studies at term-equivalent age led to the assumption that adverse neurodevelopmental outcome in preterm infants can be primarily attributed to disturbed glial maturation and neural connectivity, rather than to cell death alone [112].

Due to the advances in neonatal intensive care, cystic focal lesions leading to cerebral palsy in preterm infants have become less common. The predominant neuropathological hallmark of the encephalopathy of prematurity is a more subtle and diffuse type of damage [113–116]. Perinatal WMI has its peak incidence during the period of extensive OL migration and maturation [117]. In the human brain, the predominant stage of the OL lineage present during this vulnerable period is the premyelinating OL whereas mature OLs become abundant after term [118–120]. The accumulation of superoxide and the generation of ROS are detected as early as 2 h after oxygen exposure in OLs in vitro. Primary pre-OLs are susceptible to hyperoxia-induced cell death via caspase-dependent pathways whereas mature OLs are resistant, which suggests that the immature brain is more susceptible to oxygen toxicity [48, 121]. Experimentally, hyperoxia exposure to rodents on P6 leads to a significant increase in OL cell death, resulting in hypomyelination, detected via diminished expression of myelin basic protein (MBP) on P11 [58, 60, 64]. In this context, the effect of oxygen on myelination is also dependent on the neurodevelopmental stage, since hypomyelination in rat brains also occurs after 24 h of hyperoxia on P3 whereas oxygen exposure on P10 does not alter cerebral MBP levels (Fig. 3) [48]. In mice exposed to 48 h of hyperoxia on P6, hypomyelination can be detected from P8 to P12. Interestingly, the reduction of MBP expression seems to be transient in this model, with a compensation to control levels 1 week after the insult, possibly due to the fact that there is a hyperoxia-induced decrease in the OL population, followed by a compensatory increase in total and mature OLs [110]. However, myelin composition seems to be altered up to adult age, with a reduction of specific myelin components [122]. Despite the apparent delay in white matter maturation with the subsequent recovery of the glial population, the disruption in OL development and white matter maturation during a critical period of vulnerability leads to long-term deficiencies in the organisation and integrity of these cells. These findings are underlined by a marked reduction in diffusivity on MRI in the adult mouse brain, demonstrated by decreased fractional anisotropy and an increased radial diffusion coefficient [63, 110]. Similar results have been found in the corpus callosum and external

Fig. 3. Figure was taken from Gerstner et al. [48]. **a** Pre-OL and mature OL cell viability, measured by LDH release, after 12 h of exposure to 80% O₂ (means $\pm$ SEM of 3 independent experiments). *** $p < 0.001$, Student *t* test, hyperoxia (grey bar) vs. normoxia (white bar) for each maturation stage. **b–e** Representative phase-contrast photomicrographs of primary OLs after hyperoxia exposure. After exposure to 21% O₂, pre-OLs (**b**) and mature OLs (**d**) are intact without signs of apoptosis. Hyperoxia treated pre-OLs (**c**) show a complete loss of processes, cell shrinkage, plasma membrane blebbing, and nuclear condensation. Mature OLs do not show apoptosis after incubation in 80% O₂ for 12 h (**e**).

capsule of young and adult rat brains after 24 h of hyperoxia on P6, leading to altered white matter structures [58, 60, 64]. Hyperoxia also caused ultrastructural changes in the white matter, with a reduction of myelin thickness, abnormal myelin loops, and decreased axonal calibre, as well as the disruption of axon-OL integrity, which resulted in subsequent functional axonopathy in the corpus

callosum of mice exposed to 48 h of hyperoxia on P6 [122].

Since astrocytes as part of the neuroglia have been shown to modulate CNS damage and repair, their expression and regulation might interfere with OL function and survival [123, 124]. It has been demonstrated that hyperoxia does not affect the survival or proliferation of astro-

cytes in vivo, but modifies glial fibrillary acidic protein (GFAP) and glutamate-aspartate transporter (GLAST) expression, indicating altered glutamate homeostasis. Furthermore, cultured astrocytes exposed to hyperoxia show a reduced capacity to protect OL progenitor cells against the toxic effects of exogenous glutamate [110]. In summary, hyperoxia has an impact on OL survival and differentiation in the rodent brain, leading to impaired myelin production up to adult age, potentially contributing to an adverse neurodevelopmental outcome.

Effects of Hyperoxia on Neurons and Neuronal Plasticity

Besides diffuse WMI, a reduction of cortical grey matter volume is observed in most survivors of preterm birth [125]. The detrimental effect of neonatal hyperoxia on neuronal survival and differentiation has been described previously. Oxygen exposure in rodents from birth until P5 leads to an increase in neuronal apoptosis as well as density loss in various regions of the developing rat brain [69]. Moreover, hyperoxia administered for 24–48 h on P6 seems to decrease the total number as well as the proliferation of progenitors and immature and mature neurons [126]. Hippocampal and cerebellar volumes are reduced on MRI volumetry in 14-week-old mice after chronic exposure to hyperoxia from P2 to P14 [126]. To function properly, the CNS has to develop sufficient formation of neuronal connectivity. Synaptic plasticity is the ability of synapses to modify transmission in strength or efficacy, and plays a critical role in the conduction of impulses and therefore numerous neuronal processes. Recently, it was shown that acute, subacute, and long-lasting reductions in the expression of genes involved in synaptic plasticity regulation was induced when 6-day-old rats were exposed to 24 h of hyperoxia [60]. Therefore, hyperoxia seems to interfere with neuronal cell survival and differentiation, and influences neuronal connectivity in the rodent. Together with WMI, impaired neuronal networks may be responsible for changes in long-term neurocognitive development.

Effects of Hyperoxia on Changes in the Brain Proteome

To identify intracellular pathways engaged in a pathological modulation of maturation processes, acute (P7) and long-term (P14 and P35) changes of the brain proteome in mice subjected to high oxygen levels for 12 h on P6 have been studied. Kaindl et al. [127] revealed acute brain protein alterations after treatment with high oxygen levels. These indicate that vesicle trafficking (e.g.,

synapsin and pacsin), cell growth and differentiation (e.g., hnRNP and EF1), neuronal migration, and axonal arborisation (e.g., TUC-2/4, GAP43, and doublecortin) are impaired. Late protein changes on P35 suggest long-term/chronic disruption of cytoskeletal organisation, intracellular transport, synaptic function, and energy metabolism.

Functional Outcome

Clinical data confirms that oxygen levels in preterm infants may influence the short-term neurological outcome [49]. However, data from clinical studies has to be awaited in the future, especially for the long-term outcomes of the teenage and adult population. Moreover, in the clinical setting, the complex phenotype and multiple origins of pathologic conditions in premature infants have to be kept in mind, leaving the exact aetiology for neurodevelopmental impairment in these patients somewhat unclear. Hence, whether the behavioural and cognitive deficits observed in former preterm infants are related to hyperoxia cannot be concluded. However, experimental investigations with animal models may provide further evidence for altered neurodevelopmental outcomes. To date, only a few experimental studies have addressed this question. Behavioural testing in mice exposed to 48 h of hyperoxia revealed impaired motor activity in running wheels, starting at adolescence. Subsequently, the adolescent mice had significantly higher values for maximum and mean velocity in regular wheels than controls. In complex running wheels, however, maximum velocity decreased in animals after hyperoxia, compared to controls. The hyperoxia thus caused hyperactivity and motor coordination deficits in adolescent and young adult mice [63]. Long-term exposure of newborn C57BL/6 mice to 85% oxygen, from P2 to P14, revealed a worse performance in the water-maze and novel-object-recognition tests compared to animals exposed to room air [128]. Recently, neurobehavioural testing was performed in adolescent and adult rats after exposure to 24 h of hyperoxia on P6. In this model, general motor activity was not affected by oxygen application. However, behavioural changes were detected in the Barnes maze and novel-object-recognition tests compared to the control group, indicating long-lasting deficits in spatiotemporal learning and memory after hyperoxia treatment [60, 64].

Therapeutic Approaches

Despite its dangers and the effects on the developing organism, the use of supplemental oxygen cannot always

be avoided in the care of preterm infants and sick neonates. Predominantly in ELBW infants with underdeveloped lungs, a higher alveolar PO₂ are needed. However, excessive O₂ tensions occur in the brain when care providers increase the FiO₂ more than is necessary to compensate for pulmonary deficits during certain time periods. Before discussing therapeutic approaches, we aim to emphasise the need for the real-time monitoring of brain oxygen levels in routine NICU settings to prevent hyperoxia [129–131]. Technical advances in this field should receive higher priority than solely focussing on the discovery of therapies treating hyperoxia-induced injury. There is an urgent need to optimise oxygen therapy and search for strategies and adjunctive therapies that counteract oxygen toxicity.

For preterm infants, evidence is emerging that addresses the continued uncertainty regarding the optimal range of oxygen saturation levels and therefore oxygen supply. Between 2005 and 2007, five randomised controlled trials (RCTs) were conducted in order to identify the optimum saturation targets for extremely preterm infants. All trials examined the efficacy and safety of supplemental oxygen to target arterial oxygen saturations of 85–89% compared with 91–95%. In the US Surfactant Positive Airway Pressure and Pulse Oximetry Trial (SUPPORT), severe ROP was reduced but mortality was increased in the lower-saturation target group. In the SUPPORT and also in the Canadian Oxygen Trial (COT), there was no difference between the two groups in the combined outcome of death or neurodevelopmental impairment at 18 months [132–134]. In the two-trial BOOST-II study, conducted in Australia and the UK, non-significant higher rates of death or disability were found 2 years after targeting an oxygen saturation of 85–89% compared with 91–95%. However, the use of the lower target significantly increased the risks of this combined outcome and of death alone in the post hoc combined analyses [135].

Moreover, neurodevelopmental testing at the corrected age of 18 months may not be predictive for cognitive, behavioural, and neuropsychiatric development, as is frequently observed later in the life of extremely preterm infants. Interestingly, brain disturbances in children with congenital heart disease have a remarkable similarity to those found in preterm infants [136]. Several outcome studies of patients with congenital heart disease showed that, in early childhood, motor development seems to be more affected than cognitive development [137, 138]. This seems to change when the child gets older, with improvements in motor scores, behavioural problems, and learning difficulties [139]. Data on the long-term conse-

quences of restrictive oxygen supplementation until school age is lacking. Moreover, based on the results of these studies, it is impossible to make definitive conclusions about the duration and percentage of oxygen treatment.

Beside oxygen toxicity, a variety of consecutive insults affects brain development at different time points that are responsible for injury and adverse neurodevelopmental outcomes in preterm infants. Classical therapies have targeted individual pathways during the early phases of injury. However, regenerative therapies such as with growth factors may also enhance cell proliferation, differentiation, and migration over time. Possibly, a combined treatment with both strategies may reduce cell death and enhance repair mechanisms and/or the formation of new cells. In the clinical context, it is impossible to separate the different pathophysiological components of adverse neurodevelopmental outcomes. Therefore, experimental animal models of hyperoxia have been used to identify possible neuroprotective options. Several neuroprotective substances identified in other injury models have been investigated in experimental studies on hyperoxic brain damage. Some of these promising treatments, e.g., erythropoietin (Epo), have already made their way into clinical investigations. We will now review the current experimental evidence for neuroprotection in hyperoxia-induced brain injury. The major interest in recent years has been the evaluation of neuroprotective strategies potentially applicable in young infants.

Hormones

Endogenous hormones, like Epo or female sex steroids, have been shown to be neuroprotective. For supplementation, Epo is used in its recombinant form (rEpo). Studies on adult and also developmental brain injury models suggest that there are similar molecular mechanisms involved in hyperoxia-induced injury to the immature brain, which potentially can be targeted by rEpo. This compound has a long track record of use in preterm infants to prevent anaemia of prematurity, and it has been approved by the US FDA for clinical use. Erythropoiesis was considered originally to be the sole physiological action of rEpo. This premise was changed via the knowledge that rEpo and its receptor are expressed in several organs (including the CNS), and the subsequent discovery of its neuroprotective properties in ischemic stroke, traumatic brain injury, spinal-cord injury and perinatal asphyxia [140]. Research into the immature brain has identified numerous pathways influenced by this hormone, many of which are significant for neuroprotective effects on de-

veloping brain tissue [141]. However, larger doses of rEpo are required to obtain the desired neuroprotective effect [140].

In the rodent model of hyperoxia-induced brain damage on P6, a single treatment with 20,000 IE/kg of rEpo was recently shown to result in the long-lasting improvement of neurocognitive development, especially memory function, up to the adolescent and adult developmental stage [60]. Several neuroprotective mechanisms possibly underlying this effect have been identified. rEpo induces a significant reduction of the extent of apoptotic cell death and pro-apoptotic factors [127, 142]. This effect has also been confirmed in 5-day-old rodents subjected to hyperoxia from birth until P5 [142]. Moreover, rEpo counteracts the oxygen-induced regulation of autophagy activity proteins [78]. In parallel, rEpo inhibits most of the brain proteome changes observed when hyperoxia is applied exclusively, demonstrated on 2-dimensional electrophoresis and mass spectrometry [127]. Analysis of its molecular mode of action suggests that rEpo generates its protective effect against oxygen toxicity through a reduction of oxidative stress, pro-inflammatory mediators (i.e., IL-1 β and IL-18), matrix metalloproteinases, a restoration of the hyperoxia-induced increased levels of caspases and decreased levels of neurotrophins, and by limiting the stressor-inducible changes in both HO-1 and cholinergic functions [61, 72, 127]. Since hyperoxia induces transient hypomyelination as well as long-lasting structural white matter changes, and it has been detected in other injury models of the mature and immature brain that rEPO is protective, the impact of rEpo on OLs and myelination was recently investigated. Single-dose rEpo application resulted in a reduction of OL degeneration but did not influence myelination or white matter development [60]. As these findings differ from other injury models and the clinical findings described below, different dosage regimes and the multiple origins of brain injury in neonates have to be kept in mind. rEpo treatment does, however, reverse the hyperoxia-induced reduction of genes involved in synaptic plasticity which is thought to be important for memory function [60].

Since rEpo has been used safely in the treatment of preterm infants, clinical studies have tried to evaluate the safety and neuroprotective properties of high-dosage regimes. Several studies revealed a good tolerance for high Epo doses administered to neonates, reaching plasma levels found to be neuroprotective in rodents [143–149]. Moreover, rEpo application seems to improve white matter integrity (assessed by MRI) and neurocognitive outcomes (in retrospective and prospective investigations)

[147, 148, 150–153]. Other studies did not reveal any positive effects of rEpo therapy [154]. This might again be due to different doses and application regimes, ranging from high-dose application for a few days to lower doses for several weeks. Furthermore, developmental end points have to be considered in clinical studies, i.e., toddlers versus school-aged children. Future studies should focus on finding the optimal dosage in the setting of neonatal brain damage, further investigating the high potential of rEpo as a neuroprotective option for preterm infants.

The female hormone oestrogen (oestradiol [E2]) also has neuroprotective properties in models of *in vitro* and *in vivo* neurodegeneration in the adult and developing brain. These properties result from the activation of oestrogen receptors and cross-talk with the intracellular signalling pathways that are also activated by neurotrophins, i.e., the ERK1/2 and PI3K-Akt pathways. In addition, antioxidant properties and the modulation of NOS have been assigned to 17 β -oestradiol. E2 also has anti-inflammatory properties by reducing microglial activation and the iNOS-mediated immune response, and it produces several pro-inflammatory mediators including metalloproteinases, prostaglandin E2 (PGE₂), and cyclooxygenase 2 (COX-2). Furthermore, profound effects on the function and plasticity of the brain, and the proliferation, differentiation, and migration of neurons are controlled by E2 [155, 156].

During the last trimester of pregnancy, E2 plasma levels increase up to 15,000 pg/mL in the placenta. Both mother and foetus are exposed to the same increasing levels. At birth, the levels of E2 decrease by a factor of 100 within 24 h in the mother (150 pg/mL) and by a factor of 1,000 in the neonate (15 pg/mL). Premature infants experience this hormone deprivation and simultaneous increase of the oxygen tissue tension much earlier than infants born at term [157]. A single intraperitoneal injection of E2 provides significant dose-dependent protection against oxygen-induced apoptotic cell death in a neonatal rat model. Treatment with E2 prevents hyperoxia-induced pro-apoptotic Fas upregulation and caspase-3 activation. E2 antagonises the hyperoxia-induced inactivation of ERK1/2 and Akt, essential kinases of the MAPK and PI3K pathways that promote cell survival [158, 159]. In addition, a protective effect of E2 was also shown in immature neurons [160–162] and astroglial cells [163, 164]. Therefore, maintaining placental E2 plasma levels may be effective in protecting neonates from brain injury.

Besides the early disruption of foetal E2 supply through the placenta after premature birth, the endogenous steroid sex hormone progesterone is also reduced. However, in cultured C8-D1A astrocytes exposed to 80% oxygen for 24–72 h, there was no protective effect regarding the death or malfunction of these cells. Hyperoxia led to the downregulation of the progesterone receptors PR-AB and PR-B, which possibly explains the lack of efficacy [165]. Still, progesterone might protect other cell types in the immature brain.

E2 replacement therapy in ELBW infants has been introduced in some centres with the goal of improving bone mineralization, and no adverse side effects have been observed so far. However, no impact on the prevention of death or development of BPD has been found [166]. One RCT compared the use of E2 and/or progesterone with placebo or no treatment in 30 preterm infants (<30 weeks gestation). The primary outcome measures were neonatal mortality and medium-term neurodevelopmental effects. There was no significant effect of E2 and progesterone replacement on the outcomes of mortality or neurodevelopmental disability in survivors followed. No adverse effects of sex steroid replacement on short- or long-term outcomes were detected [167].

In another randomised study, 83 preterm infants with a birth weight <1,000 g and a gestational age <29 weeks were administered E2 and progesterone [168]. No significant effect was found in preventing BPD or death in this extremely preterm population. Follow-up at 5 years of age did not reveal any differences between the replacement and placebo groups on the Gross Motor Function Classification Scale, or with regard to the presence of paresis, cerebral palsy, or spasticity [169].

In summary, E2 is a well-known neuroprotective compound whose efficiency has been shown in numerous experimental studies. However, preterm birth creates a specific hormonal milieu, found solely in humans and higher primates [170]. The role of sex steroids and their receptors has to be explored in more detail with respect to the developing organism. More data is needed on the safety and feasibility of gestational hormone supplementation in the neonatal period. Sufficiently powered RCTs are required to determine whether the administration of E2 confers clinically significant benefits, or has risk factors for the preterm infant.

Neuroprotection via Immunomodulation

A potential drug for protecting the white matter is minocycline, a tetracycline-antibiotic used for treating infections. Its neuroprotective capacity has been demon-

strated in different models of the immature brain, like hypoxia-ischemia and perinatal inflammation/infection [171–173]. The beneficial effects of minocycline have been attributed to its inhibition of microglial activation which occurs under hyperoxic conditions. Minocycline administration in 6-day-old rats exposed to hyperoxia resulted in decreased apoptotic cell death, improved proliferation, and maturation of OL progenitor cells. In this setting, minocycline decreased the number of IBA1-positive cells (representing activated microglia) and the hyperoxia-induced release of IL-1 β . It was concluded that this compound exhibits a dual effect via the direct protection of OLs and the inhibition of microglial cells [174].

Fingolimod (FTY720) is a sphingosine-1-phosphate (S1P) analogue and receptor modulator clinically used in the therapy of relapsing-remitting multiple sclerosis. Although the exact mechanisms of action are unclear, it is suggested that the degradation of S1P receptors inhibits immune cell migration into the CNS [175, 176]. Independent of peripheral immune modulation, FTY720 crosses the blood-brain barrier and can directly modulate CNS cells like microglia and OLs, which express S1P receptors [177]. After 24 h of hyperoxia in 6-day-old rats, FTY720 improved the neurocognitive outcome up to adolescence and adulthood. The FTY720 treatment also led to a reduction of S1P receptor-1 expression, oxidative stress, microglia activation, and the associated pro-inflammatory cytokine production. Oxygen-induced hypomyelination, OL degeneration, and microstructural white matter changes were restored. FTY720 is a promising candidate for neuroprotection in hyperoxic brain injury [64].

Stem Cells

Stem cells are undifferentiated cells that differentiate into tissue-specific cell lines under certain circumstances. Depending on their origin, these cells can be neuronal, mesenchymal, or haematopoietic. Mesenchymal stem cells (MSCs) seem to have relevant neuroprotective properties in experimental animal injury models of the brain [178–180]. Newborn Sprague-Dawley rats exposed to hyperoxia for 14 days received MSCs (5×10^5 cells) intratracheally on P5. The pups treated with MSCs showed significantly fewer hyperoxia-induced apoptotic cells in the dentate gyrus and reduced the hypomyelination [181]. Since experimental information is limited to one publication and the underlying mechanisms are unclear at this point, we await further studies for confirmation of the observed effects and identification of the potential molecular mechanisms involved.

CNS-Active Substances

Several other drugs used for treating CNS disorders have been tested in neonatal hyperoxic brain injury. Dexmedetomidine, a selective agonist of α_2 receptors, has sedative, anxiolytic, analgesic, and anaesthetic properties [182–184]. Its neuroprotective effects have been widely described [185, 186]. In 6-day-old Wistar rat pups, dexmedetomidine application significantly reduced hyperoxia-induced neurodegeneration and IL-1 β mRNA and protein levels after 24 h of hyperoxia. Moreover, pretreatment with dexmedetomidine normalises the reduced/oxidised glutathione ratio as well as reduced levels of lipid peroxidation [187]. Whether this compound is protective in other experimental models of brain injury needs to be further investigated.

A substance frequently used in neonatal care for the prevention of apnoea and for respiratory stimulation is caffeine [185, 186]. Interestingly, large, placebo-controlled, multi-centre trials involving caffeine use have revealed a reduction of cerebral palsy and neurodevelopmental impairment [188, 189]. In neonatal animal models of chronic hypoxia, caffeine has been shown to ameliorate hypomyelination as well as ventriculomegaly [190]. In 6-day-old rodents, the administration of 10 mg/kg caffeine led to a reduction of apoptosis and the prevention of neuronal progenitor cell loss at 24–48 h of hyperoxia exposure [126]. Since caffeine is already safe to use in the care of neonates, further studies are awaited to confirm these effects.

Another promising neuroprotective mechanism recently investigated in hyperoxic brain injury is acetylcholinesterase (AChE) inhibition. AChE hydrolyses acetylcholine and is widely expressed in the nervous system, more specifically in the cholinergic and cholinceptive neurons and the neuromuscular junctions [191]. AChE seems to be altered by stress conditions and cell death, but enhanced AChE levels can elevate apoptosis [191–195]. Moreover, AChE is linked to the development of the brain, e.g., cell growth and adhesion, neuronal damage, and immune response regulation [191, 195–199]. Acetylcholine, on the other hand, seems to reduce pro-inflammatory cytokines [197, 200, 201]. In hyperoxia-induced neonatal brain injury, the upregulation of AChE has been detected [72]. After 12–24 h of hyperoxia exposure to 6-day-old rat pups, pretreatment with the AChE inhibitors physostigmin (100 μ g/kg) and donepezil (200 μ g/kg) resulted in the reduction of AChE activity, and IL-1 β , TNF- α mRNA and protein expression as well as the amelioration of oxidative stress and neuronal cell death [102].

Dextromethorphan (DM) is an antitussive agent widely used in paediatric care [202, 203]. DM has already proven its therapeutic potential in different neonatal brain damage models like excitotoxicity and hypoxia-ischemia [204–206]. It has numerous neuroactive properties including high-affinity σ_1 receptor agonism, low-affinity N-methyl-D-aspartate receptor (NMDAR) antagonism, and voltage-gated calcium channel antagonism, with additional anti-inflammatory and antioxidative qualities [207]. In 6-day-old rats exposed to 24 h of hyperoxia, a single dose (5 or 25 μ g/kg of body weight) of DM significantly reduced apoptosis on immunohistochemistry. Moreover, the cell viability of immature OLs (OLN-93) subjected to hyperoxia was dose-dependently preserved, indicating its protective effects *in vivo* as well as *in vitro* [208].

Zonisamide (1,2-benzisoxazole-3-methanesulphonamide) is an anticonvulsive agent, which blocks voltage-dependent sodium and T-type calcium channels [209]. The neuroprotective effect of this drug has been demonstrated in neonatal hypoxic-ischemic brain damage [210]. Zonisamide seems to inhibit excitotoxic pathways, decrease extracellular glutamate accumulation, reduce free radicals, and inhibit NOS activity [210, 211]. In rats subjected to a hyperoxic environment, from birth until P5, daily treatment with zonisamide (75 mg/kg body weight) results in the augmentation of neurons as well as reducing neuronal death in different brain regions [212]. Zonisamide may therefore be a promising candidate, especially for preterm infants who are diagnosed with seizures.

Topiramate is an antiepileptic drug working as a potential neuroprotective agent against hypoxic-ischemic and hyperoxic brain injury. Topiramate prevents seizures by inhibiting neuronal excitability by blockade of glutamate receptors [213]. After carotid artery ligation in the neonatal rat, topiramate significantly reduced neuronal death by the inhibition of glutamate receptor activity [214]; it also reduced hypoxic-ischemic-induced neuronal apoptosis in newborn piglets [215]. Topiramate has been found to be protective against hyperoxia from birth until P5 in neonatal rats. Histopathological examination showed that topiramate significantly diminished apoptosis in the CA1 region and dentate gyrus of the hippocampus [216].

Antioxidants

There are many antioxidants that have been investigated in both preterm and full-term hypoxic-ischemic injury, and scavengers such as melatonin and allopurinol have shown promise [217–219]. Allopurinol has anti-inflammatory properties in hyperoxia-induced lung injury

Fig. 4. Patterns of hyperoxic injury mechanisms and neuroprotective strategies.

as demonstrated by a reduction of the alveolar neutrophilic response [218]. Melatonin has been shown to significantly reduce oxidative stress in adult brain tissue samples [219]. However, to determine its effects on the immature, hyperoxia-exposed brain, studies are still pending.

Experimental Drugs

Different substances invented to interfere with endogenous signalling cascades might also be interesting candidates to protect against oxygen-induced brain injury.

The inhibition of key players of the apoptotic cascade appears to be a promising strategy for neuroprotection. Besides the receptor-mediated, extrinsic apoptotic pathway, hyperoxia-mediated neurodegeneration in the developing brain is supported by intrinsic apoptosis, suggesting that the development of highly selective caspase inhibitors will represent a potential useful therapeutic strategy in prematurely born infants. Injection of the selective caspase-8 inhibitor (TRP801), a downstream effector caspase in the receptor-mediated apoptotic pathway, subsequently blocked caspase-3 cleavage and conferred neuroprotection in 6-day-old rats exposed to 24 h of hyperoxia [59]. Elevated oxygen levels also trigger a marked increase in active caspase-2 expression, resulting in an initiation of the intrinsic apoptotic pathway, involving the mitochondrial route, with the upregulation of key proteins, namely, cytochrome c, Apaf-1, and AIF. A single treatment with TRP601 at the beginning of hyperoxia

reversed the detrimental effects in this model [71]. Hyperoxia-mediated neurodegeneration is supported by intrinsic apoptosis, suggesting that the development of highly selective caspase inhibitors may represent a potentially useful therapeutic strategy.

Since oxidative stress is a major mechanism implicated in a variety of neurodegenerative diseases, antioxidative medication can be protective. The lipid-metabolising enzyme 12/15-lipoxygenase (12/15-LOX) mediates cell death in both neurons and OLs. Once activated, 12/15-LOX generates lipid hydroperoxides that serve to further amplify oxidative stress [220]. In hyperoxia-induced cell death in OL cultures, the effects of the 12-LOX inhibitors AA-861 and *N*-benzyl-*N*-hydroxy-5-phenylpentanamide (BMD-122 and BHPP) were effective in blocking cell death. In addition, the LOX inhibitor baicalein, which also has antioxidant properties, exhibited a protective effect against hyperoxia-mediated OL cell death [48].

Figure 4 provides a schematic illustration of patterns of hyperoxic injury mechanisms and neuroprotective strategies.

Conclusions

Hyperoxia causes oxidative stress and contributes to the pathogenesis of injury in the preterm as well as the full-term brain. During the critical time period of brain

development, the immature CNS is particularly vulnerable to this type of stress. From current experimental evidence, it may be hypothesised that oxygen causes cell death and profoundly alters maturational processes. Multiple cell types such as neurons, OLs, astrocytes, and microglia cells are affected. Behavioural studies on animals have revealed effects such as motor hyperactivity and cognitive impairment, similar to those observed in former preterm infants at school age. Furthermore, characteristic MRI findings in hyperoxia-exposed rodents show-

ing reduced hippocampal size and white matter abnormalities resemble the images of prematurely born infants at term.

Therapeutic efforts aiming at defining the optimal oxygen saturation and the development of adequate monitoring systems are highly warranted. Furthermore, in situations where oxygen supplementation cannot be avoided, the development of adjunctive therapies is a major challenge for current experimental research.

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