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Brain susceptibility to oxidative stress in the perinatal period

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Abstract

Oxidative stress (OS) occurs at birth in all newborns as a consequence of the hyperoxic challenge due to the transition from the hypoxic intrauterine environment to extrauterine life. Free radical (FRs) sources such as inflammation, hyperoxia, hypoxia, ischaemia-reperfusion, neutrophil and macrophage activation, glutamate and free iron release, all increases the OS during the perinatal period. Newborns, and particularly preterm infants, have reduced antioxidant defences and are not able to counteract the harmful effects of FRs. Energy metabolism is central to life because cells cannot exist without an adequate supply of ATP. Due to its growth, the mammalian brain can be considered as a steady-state system in which ATP production matches ATP utilisation. The developing brain is particularly sensitive to any disturbances in energy generation, and even a short-term interruption can lead to long-lasting and irreversible damage. Whenever energy failure develops, brain damage can occur. Accumulating evidence indicates that OS is implicated in the pathogenesis of many neurological diseases, such as intraventricular haemorrhage, hypoxic-ischaemic encephalopathy and epilepsy.

Keywords

Antioxidants, brain injury, free radicals, oxidative damage

History

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Introduction

Oxidative stress (OS) occurs when the production of free radicals (FRs) exceeds the capacity of antioxidant defences. It represents an imbalance between the production of reactive species and the biological ability to readily detoxify the reactive intermediates or to repair the resulting damage. Each cell is characterised by a particular concentration of electrons (redox state) stored in many cellular constituents, and the redox state of a cell with its oscillation determines cellular functioning [1].

Under normal conditions, the redox environment of cells is kept within a narrow range. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and FRs that damage all cell components, including proteins, lipids, polysaccharides and DNA (fragmentation, apoptosis, base modifications and strand breaks) [2]. Some reactive oxidative species can even act as messengers through a phenomenon called redox signalling. Reactive oxygen and nitrogen species change cellular responses through diverse mechanisms: at low levels, they are signalling molecules, and at high levels, they can damage organelles, particularly the mitochondria with further amplification of damage. A certain amount of OS is required to allow the normal progression of embryonic and foetal growth [3]. OS has been implicated in the regulation of reproductive processes in both animal and human, such as follicular development, ovulation, fertilisation, embryogenesis, placental differentiation and growth. In contrast, OS has emerged as a likely promoter of several pregnancy-related disorders, such as miscarriages, embryopathies, preeclampsia, foetal growth restriction, preterm labour and low birth weight. Hence, the paradox of aerobic life, or the ''Oxygen Paradox'', explains why higher eukaryotic aerobic organisms cannot exist without oxygen and without OS, even if oxygen and FRs in general are dangerous to their existence.

Oxidative injury and the associated mitochondrial dysfunction may result in energy depletion, accumulation of cytotoxic mediators and cell death. Understanding the interface between stress adaptation and cell death is important to clarify redox biology and disease pathogenesis.

OS occurs at birth in all newborns as a consequence of the hyperoxic challenge due to the transition from the hypoxic intrauterine environment to extrauterine life. FR sources such as inflammation, hyperoxia, hypoxia, ischaemia-reperfusion, neutrophil and macrophage activation, glutamate and high free iron release, all increases the OS during the perinatal period. Hypoxia can lead to the shift from aerobic to anaerobic metabolism leading to increased levels of lactic acid and FRs [4]. Increased susceptibility to infection and inflammation, as well as the presence of free iron in the plasma and tissue of premature infants also contribute to augmenting OS vulnerability in the early phase of life. In addition, newborns and especially preterm infants have

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reduced antioxidant defences and are not able to counteract the harmful effects of FRs which promote cellular, tissue and organ damage and lead to the so called ''free radicals related disease'' of newborns [5].

It has been hypothesised that OS is also involved in a number of pathological conditions in adults involving the cardiovascular, pulmonary, renal, gastrointestinal, hepatic and neurological systems, as well as metabolic and inflammatory diseases [6].

Oxidative stress during brain development

In many animals, especially in human beings, the brain undergoes substantial quantitative and qualitative changes that occur primarily, or solely, during development. These include cell division, differentiation and migration, axonal and dendritic proliferation, synaptogenesis, myelination, programmed cell death and formation of neuronal networks. These processes require a complex network of signalling molecules, ion channel cerebral expression, receptor maturation and growth factor synthesis [7].

Due to its growth, the mammalian brain can be considered as a steady-state system in which ATP production matches ATP utilization. Energy metabolism is central to life because cells cannot exist without an adequate supply of ATP. The brain is particularly sensitive to any disturbances in energy generation and even a short-term interruption can lead to long-lasting and irreversible damage. Every time energy failure develops, brain damage can occur.

Accumulating evidence indicates that OS is implicated in the pathogenesis of many neurological diseases, such as intraventricular haemorrhage (IVH) [8], hypoxic-ischaemic encephalopathy (HIE) [9] and epilepsy [10].

The particular vulnerability of the developing brain to hypoxic-ischaemic injury may be related to intrinsic regional metabolic factors. First of all, the cerebral metabolic rate for glucose and for oxygen, energy consumption and cerebral blood flow in the developing brain are higher than in the matured brain. These biochemical changes are accompanied by modifications in the mitochondrial structures and functional activity, i.e., the number of mitochondria per cell, mitochondrial protein and respiratory enzyme content, and mitochondrial matrix density [11]. The telencephalic white matter, especially in the depths of the sulci, represents a border zone of blood supply between major cerebral arteries. The relative sparing of cerebral grey matter is explained by the presence of numerous leptomeningeal anastomoses among major cerebral arteries, a characteristic feature in the foetal brain. The high concentration of unsaturated fatty acids in the neonatal brain predisposes the generation of FRs and the propagation of OS. Polyunsaturated fatty acid constituents of membrane lipids in the white matter are highly susceptible to FR damage. FR attacks on immature myelin sheaths lead to lipid peroxidation and lipid peroxides are themselves FRs [12].

Antioxidant defences during hyperoxic challenge are impaired during neonatal life. Specifically, superoxide dismutase, catalase and glutathione peroxidase antioxidant enzyme systems are less concentrated and show less activity in the immature brain than in the matured one [13]. Transiently increased density and a wide distribution of glutamate receptors in the developing brain could amplify brain injury due to hypoxic damage [14].

One of the aspects of cell damage in the developing brain is related to the pivotal role of mitochondria in cell metabolism under normal conditions and after hypoxia-ischaemia (HI). Following a hypoxic stimulus there is a certain degree of swelling and calcium deposit inside the mitochondria [15], which leads to chromatin condensation, thus triggering apoptosis. Cerebral apoptosis starts with cytochrome C translocation from mitochondria, followed by caspase 9 activation and then caspase 3 activation. Many apoptosis-related factors are upregulated in the immature brain including caspase-3, Apaf-1, Bcl-2 and Bax [16]. In the developing brain, NMDA receptor activation depresses mitochondrial respiration and induces apoptosis, a phenomenon that is not seen in the adult brain (the so-called NMDA-paradox) [14]. Subtype is essential for the antiapoptotic signalling integrity of amino-acid receptors of the NMDA.

The developing brain is prone to produce FR from oxygen and NO; they operate intramitochondrial protein nitrosilation, which triggers cell death and/or apoptosis. It is thus plausible that the intramitochondrial scavenging system in the developing brain fails to detoxify nitrogen and oxygen FRs [17]. It is clear that mitochondria play crucial roles in the activation of apoptotic mechanisms; they are both initiators and targets of OS. In a prospective study conducted on 90 newborns (>32 GA) with various stages of HIE, the authors studied glutathione peroxidase (GPX) activity in the cerebrospinal fluid in the first 48 h of life as an index of OS. They also examined the concentration of neuron-specific enolase (NSE) at 72 h of life as a marker of brain injury. Neurological outcome was assessed at 12 months of corrected GA using the Denver Developmental screening test. Interestingly, they found a correlation between GPX activity and GA, clinical stage of HIE, NSE levels and neurodevelopmental outcome [18].

It has also been suggested that the broad variation in the final effects of hypoxia-ischaemia on the neonatal brain is due to genetic factors and that there is a gender difference regarding response to HI injury, with male newborns being more susceptible to injury than females [19].

The negative effects of OS may start from intrauterine life. It has been demonstrated that OS plays a key role in some pathological conditions associated with neurological deficits (i.e., cerebral palsy, cognitive and behavioural disorders), such as intrauterine growth retardation (IUGR) [20]. A recent *in vivo* and *ex vivo* rat model of IUGR shows the delay in oligodendrocyte differentiation and myelination, likely due to bone morphogenetic protein 4 (BMP4) upregulation induced by OS. Normal myelination has been observed when abrogating BMP signalling [21].

Down syndrome originates from an extra chromosome 21 in the cellular karyotype. The superoxide dismutase (SOD) gene is localised on chromosome 21. This enzyme dismutates superoxide anion *in vivo* with the participation of catalase and glutathione peroxidase. Increased levels of 8-iso-PGF2 isoprostane, a reliable biomarker of OS, were found in the amniotic fluid of pregnancies with a Down syndrome

foetus [22]. The immature oligodendroglial cells are glutathion peroxidase and catalase deficient, so overexpression of SOD can be dangerous rather than protective. The early occurrence of OS in pregnancies with a trisomy 21 foetus and the subsequent oxidative damage as a major contributing factor in brain ageing and cognitive function decline are likely due to the overexpression of SOD due to the supernumerary chromosome. SOD is also overexpressed in the immature brain, especially under stress condition.

Brain injury in the preterm infant: intraventricular haemorrhage

IVH in very preterm infants is an acquired and common event with an enormous, potential impact on mortality, morbidity and severe long-term consequences. Haemorrhage into the germinal matrix tissues of the developing brain has been attributed to changes in cerebral blood flow to the immature germinal matrix microvasculature and to secondary periventricular venous infarction. Even though risk factors for IVH remain to be further defined, the mechanism of IVH development is multifactorial and involves a combination of vascular immaturity and haemodynamic factors (Figure 1). Recently, more detailed analyses have demonstrated the role of OS in IVH and the consequent involvement of FRs, with subsequent endothelial damage to the germinal matrix and to the white matter [23]. The most vulnerable cell populations include subplate neurons and oligodendrocyte precursors. Non-protein bound iron (NPBI) concentration in cord blood was found to be highly predictive for the risk of poor neurodevelopmental outcome [24]. NPBI indicates a low molecular mass iron form, free of high-affinity binding to transferrin and that is able to catalyse the formation of hydroxyl radical, a highly toxic molecule.

NBPI is responsible for lipid peroxidation and F2-isoprostane increase. In the perinatal period and especially among premature infants, low levels of transferrin, decreased transferrin iron-binding capacity, and low levels of ceruloplasmin and ferroxidase activity may contribute to generating NPBI in the amniotic fluid and in plasma. Neuronal membranes, which are very rich in polyunsaturated fatty acids, are highly sensitive to FR attack. In a small sample of premature infants with white matter injury, the cerebrospinal fluid 15-F2t-IsoP levels, which are markers of lipid peroxidation, were significantly increased in one-third of the subjects [25]. Vascular cell functioning is also injured by FR attack, which induces endothelial dysfunction, considered a crucial factor in the development of vascular diseases.

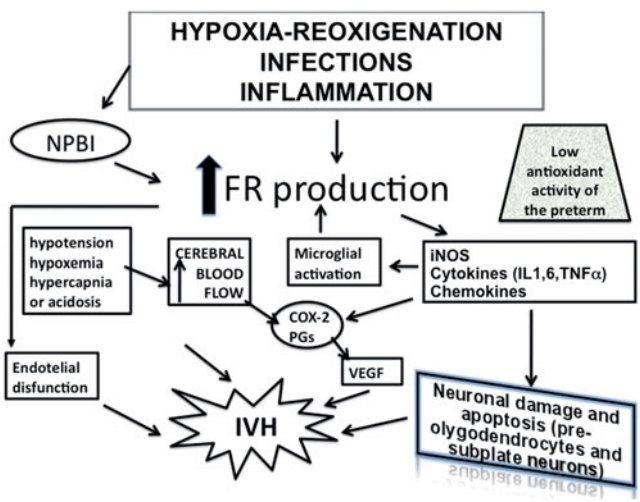

Figure 1. Mechanisms involved in intraventricular haemorrhage onset. OX2: cycloxygenase2; FR: free radicals; iNOS: inducible nitric oxide synthase; IVH: intraventricular haemorrhage; NPBI: non-protein binding iron; PGE2: prostaglandin; VEGF: vascular endothelial growth factor.

The propensity of the preterm brain to the dangerous effects of oxidative action relates not only to deficient antioxidant defences, but also to several pro-oxidant characteristics. At the most basic level, developing tissues have a high metabolic rate supported almost exclusively by oxidative metabolism, which is an excellent source of FR, and a relatively high NPBI concentration [26].

Moreover, hypoxia and ischaemia during perinatal asphyxia are among the major factors of NPBI release and brain injury in newborn infants. The reperfusion or reoxygenation phase in the immediate post-hypoxia-ischaemia period may further increase the injury [27]. Asphyxia and acidosis supply redox-cycling iron, enhancing NPBI release into plasma, causing impaired endothelial function.

Furthermore, the periventricular and the germinal matrix areas of preterm babies are richly vascularised by microvessels lacking basement membrane deposition, tight junctions and glial endfoot investiture, all of which are components of a competent blood brain barrier. Cerebral blood flow increases in response to many injuries, such as hypotension, hypoxemia, hypercapnia or acidosis. The result of this process is a haemorrhage beginning within the germinal matrix and carrying on into the ventricular system.

Blood flow decreases following ventricular distension by an acute haemorrhagic event. Venous stasis occurs within the periventricular white matter, and parenchymal venous infarction may follow. Important modulators of cerebral blood flow in the developing brain include the cyclo-oxygenase 2 (COX-2) system and prostaglandins (PGs). COX-2 expression is induced by hypoxia, hypotension, growth factors such as epidermal growth factor receptor, transforming growth factor β (TGFβ) and inflammatory modulators including IL-6, IL-1β, TNF-α and NFkappaB [28]. The resultant promote the production and release of vascular endothelial growth factor (VEGF), a potent angiogenic factor [29].

The same triggers which initiate haemorrhage into the germinal matrix also promote a cascade leading to the disruption of tight junctions, increased blood brain barrier permeability, and microglial activation within the developing periventricular white matter. These events are mediated by cytokines, VEGF and nitric oxide (NO). Finally, reactive microglia release FRs, which contribute not only to endothelial damage, but also alter haemostasis and increase anaerobic metabolism. Endothelial cell injury and dysfunction may additionally contribute to the inflammatory response and alterations in coagulation through loss of normal endothelial nitric oxide production [30]. Other potential implications of iron overload include acute impairment of endothelium-dependent flow-mediated vasodilation, as demonstrated in *in vivo* and *in vitro* studies [31,32]. Iron overload and iron-mediated FR production can also cause loss of tight junction proteins and degeneration of endothelial cells, as well as

opening of the blood-brain barrier after hypoxia-ischaemia [33]. The blood brain barrier plays a critical role in maintaining communication between the brain and peripheral tissues. Separation of endothelial tight junctions, loss of endothelial attachment to the basement membrane, endothelial blebbing and endothelial necrosis have been described in the cerebral vasculature following ischaemic injury. The presence of iron and the imbalance of redox homeostasis within the vascular end zones and border zones in the immature brain play an important role in initiating and extending cerebral epithelial injury. The progression of endothelial dysregulation can contribute to the ongoing pathogenesis of IVH. Because of their multifaceted effects on the developing vasculature, FRs are believed to play a significant role in periventricular parenchymal infarction [34].

The nature of the cells involved in OS-associated brain injury is currently unclear. Post-mortem examination of premature infants revealed that brain oxidative damage particularly targets the oligodendrocyte lineage, suggesting that these cells are most vulnerable to this type of injury [35]. Other vulnerable cell populations include the subplate neurons.

In conclusion, the physiologic qualities of the neonatal brain make its response to any insult unique. Oxidative stress presents numerous opportunities for brain injury through the formation of reactive oxygen/nitrogen species. The degree of damage depends on the region of the brain that is affected, the severity of the insult and the stage of development. The morbidity and mortality of infants, especially if preterm, are strongly affected by their ability to maintain physiologic homeostasis and to counteract the effects of FRs. The peculiar perinatal susceptibility to OS indicates that prenatal prophylactic use of antioxidants could help to prevent or at least reduce OS-related diseases in foetuses and newborns.

Declaration of interest

The authors report no conflicts of interest.

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References

1. Ostrakhovitch EA, Semenikhin OA. The role of redox environment in neurogenic development. Arch Biochem Biophys. 2012; 528:21–31.
2. Saugstad OD. Mechanisms of tissue injury by oxygen radicals: implications for neonatal disease. Acta Paediatr 1996;85:1–4.
3. Dennery PA. Role of redox in fetal development and neonatal diseases. Antioxid Redox Signal 2004;6:147–53.
4. Mishra OP, Delivoria-Papadopoulos M. Cellular mechanisms of hypoxic injury in the developing brain. Brain Res Bull 1999;48: 233–8.
5. Perrone S, Tataranno ML, Negro S, et al. Early identification of the risk for free radical-related diseases in preterm newborns. Early Hum Dev 2010;86:241–4.
6. Basu S. F2-isoprostanes in human health and diseases: from molecular mechanisms to clinical implications. Antioxid Redox Signal 2008;10:1405–34.
7. Erecinska M, Cherian S, Silver IA. Energy metabolism in mammalian brain during development. Prog Neurobiol 2004;73: 397–445.
8. Chua CO, Vinukonda G, Hu F, et al. Effect of hyperoxic resuscitation on propensity of germinal matrix haemorrhage and cerebral injury. Neuropathol Appl Neurobiol 2010;36:448–58.
9. Ten VS, Starkov A. Hypoxic-ischemic injury in the developing brain: the role of reactive oxygen species originating in mitochondria. Neurol Res Int 2012;2012:542976. doi: 10.1155/2012/542976.
10. Waldbaum S, Patel M. Mitochondrial dysfunction and oxidative stress: a contributing link to acquired epilepsy? J Bioenerg Biomembr 2010;42:449–55.
11. Erecinska M, Cherian S, Silver IA. Energy metabolism in mammalian brain during development. Prog Neurobiol 2004;73: 397–445.
12. Halliwell B. Reactive oxygen species and the central nervous system. J Neurochem 1992;59:1609–23.
13. Baud O, Greene AE, Li J, et al. Glutathione peroxidase-catalase cooperativity is required for resistance to hydrogen peroxide by mature rat oligodendrocytes. J Neurosci 2004;24: 1531–40.
14. Ikonomidou C, Bosch F, Miksa M, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283:70–4.
15. Puka-Sundvall M, Gajkowska B, Cholewinski M, et al. Subcellular distribution of calcium and ultrastructural changes after cerebral hypoxia – ischemia in immature rats. Brain Res Dev Brain Res 2000;125:31–41.
16. Ota K, Yakovlev AG, Itaya A, et al. Alteration of apoptotic protease-activating factor-1 (APAF-1)-dependent apoptotic pathway during development of rat brain and liver. J Biochem 2002; 131:131–5.
17. Peeters-Scholte C, Koster J, Veldhuis W, et al. Neuroprotection by selective nitric oxide synthase inhibition at 24 hours after perinatal hypoxia – ischemia. Stroke 2002;33:2304–10.
18. Vasiljevic B, Maglajlic-Djukic S, Gojnic M, et al. New insights into the pathogenesis of perinatal hypoxic-ischemic brain injury. Pediatr Int 2011;53:454–62.
19. Nuñez JL, McCarthy MM. Sex differences and hormonal effects in a model of preterm infant brain injury. Ann N Y Acad Sci 2003; 1008:281–4.
20. Longini M, Perrone S, Kenanidis A, et al. Isoprostanes in amniotic fluid: a predictive marker for fetal growth restriction in pregnancy. Free Radic Biol Med 2005;38:1537–41.
21. Reid MV, Murray KA, Marsh ED, et al. Delayed myelination in an intrauterine growth retardation model is mediated by oxidative stress upregulating bone morphogenetic protein 4. J Neuropathol Exp Neurol 2012;71:640–53.
22. Perrone S, Longini M, Bellieni CV, et al. Early oxidative stress in amniotic fluid of pregnancies with Down syndrome. Clin Biochem 2007;40:177–80.
23. McCrea HJ, Ment LR. The diagnosis, management, and postnatal prevention of intraventricular hemorrhage in the preterm neonate. Clin Perinatol 2008;35:777–92, vii.
24. Buonocore G, Perrone S, Longini M, et al. Non protein bound iron as predictive marker of neonatal brain damage. Brain 2003; 126:1–7.
25. Inder T, Mocatta T, Darlow B, et al. Elevated free radical products in the cerebrospinal fluid of VLBW infants with cerebral white matter injury. Pediatr Res 2002;52:213–18.
26. Papile LA, Burstein J, Burstein R, et al. Relationship of intravenous sodium bicarbonate infusions and cerebral intraventricular hemorrhage. J Pediatr 1978;93:834–6.
27. McQuillen PS, Ferriero DM. Selective vulnerability in the developing central nervous system. Pediatr Neurol 2004;30: 227–35.
28. Dammann O, Leviton A. Inflammatory brain damage in preterm newborns – dry numbers, wet lab, and causal inferences. Early Hum Dev 2004;79:1–15.
29. Kuwano T, Nakao S, Yamamoto H, et al. Cyclooxygenase 2 is a key enzyme for inflammatory cytokine-induced angiogenesis. FASEB J 2004;18:300–10.
30. Yang Y, Loscalzo J. Regulation of tissue factor expression in human microvascular endothelial cells by nitric oxide. Circulation 2000; 101:2144–8.
31. Kartikasari AE, Georgiou NA, Visseren FL, et al. Endothelial activation and induction of monocyte adhesion by nontransferrin-bound iron present in human sera. FASEB J 2006;20:353–5.

32. Rooyakkers TM, Stroes ES, Kooistra MP, et al. Ferric saccharate induces oxygen radical stress and endothelial dysfunction in vivo. Eur J Clin Invest 2002;32:9–16.
33. Won SM, Lee JH, Park UJ, et al. Iron mediates endothelial cell damage and blood-brain barrier opening in the hippocampus after transient forebrain ischemia in rats. Exp Mol Med 2011;43: 121–8.
34. Folkerth RD, Haynes RL, Borenstein NS, et al. Developmental lag in superoxide dismutases relative to other antioxidant enzymes in premyelinated human telencephalic white matter. J Neuropathol Exp Neurol 2004;63:990–9.
35. Back SA, Luo NL, Mallinson RA, et al. Selective vulnerability of preterm white matter to oxidative damage defined by F2-isoprostanes. Ann Neurol 2005;58:108–20.