Assessment of Oxidative Stress in Neonatal Brain Damage

Subjects: Pediatrics

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Oxidative stress (OS) constitutes a pivotal factor within the mechanisms underlying brain damage, for which the immature brain is particularly vulnerable. This vulnerability is caused by the abundance of immature oligodendrocytes in the immature brain, which are highly susceptible to OS-induced harm. Consequently, any injurious process involving OS within the immature brain can lead to long-term myelination impairment. Among the detrimental repercussions of OS, protein carbonylation stands out as a prominently deleterious consequence. Noteworthy elevation of protein carbonylation is observable across diverse models of neonatal brain injury, following both diffuse and focal hypoxic–ischemic insults, as well as intraventricular hemorrhage, in diverse animal species encompassing rodents and larger mammals, and at varying stages of brain development. In the immature brain, protein carbonylation manifests as a byproduct of reactive nitrogen species, bearing profound implications for cell injury, particularly in terms of inflammation amplification. Moreover, protein carbonylation appears as a therapeutic target for mitigating neonatal brain damage. The administration of a potent antioxidant, such as cannabidiol, yields substantial neuroprotective effects.

oxidative stress protein carbonylation cannabidiol brain damage newborn

1. Introduction

Neonatal-acquired brain damage is a prevalent condition affecting 1–4 per 1000 newborns [1][2]. This encompasses a spectrum of injuries, including diffuse or focal hypoxic–ischemic damage, complications arising from prematurity, as well as damage attributed to infectious diseases or central nervous system trauma [1][2]. Each year, over two million infants globally either die or survive with permanent disabling repercussions due to such injuries [2]. Despite the pressing significance of this issue, a substantive treatment for acquired brain damage in newborns remains elusive, barring therapeutic hypothermia, which benefits approximately 60% of full-term newborns with moderate to severe hypoxic-ischemic encephalopathy [1].

The inefficacy of therapeutic strategies in the neonatal population is closely tethered to the impossibility to predict the development of brain damage after a perinatal insult in the majority of cases, as well as to the intricate pathophysiology of brain damage [1][3]. Similar to other developmental stages, the foremost drivers of brain damage comprise neuroinflammation, excitotoxicity, and oxidative stress (OS), each mutually potentiated [3][4]. Notably, the role of OS is assuming prominence due to its pivotal involvement in both structural and functional impairment.

Reactive oxygen species (ROSs), primarily originating from compromised mitochondrial respiration; along with reactive nitrogen species (RNSs), primarily generated through excessive nitric oxide (NO) production; and reactive halogen species, produced after the oxidation of halogen ions by myeloperoxidase, adversely affect protein, lipid, and DNA integrity [5][6][7][8]. These agents disrupt mitochondrial energy generation and perturb the redox state irreversibly, precipitating towards an imbalance termed OS [5][6][7]. Finally, OS can lead to endoplasmic reticulum (ER) stress [9]. Since the normal functioning of the ER requires strict control of the redox environment, the surge in OS leads to functional alteration of the ER, with the consequent impairment of normal protein synthesis and activation of a variety of signaling pathways that lead to brain damage [9].

Figure 1 presents a diagram that includes the main pathways of oxidative stress generation and its consequences, including biomarkers of strategic value for the study of OS in the neonatal brain.

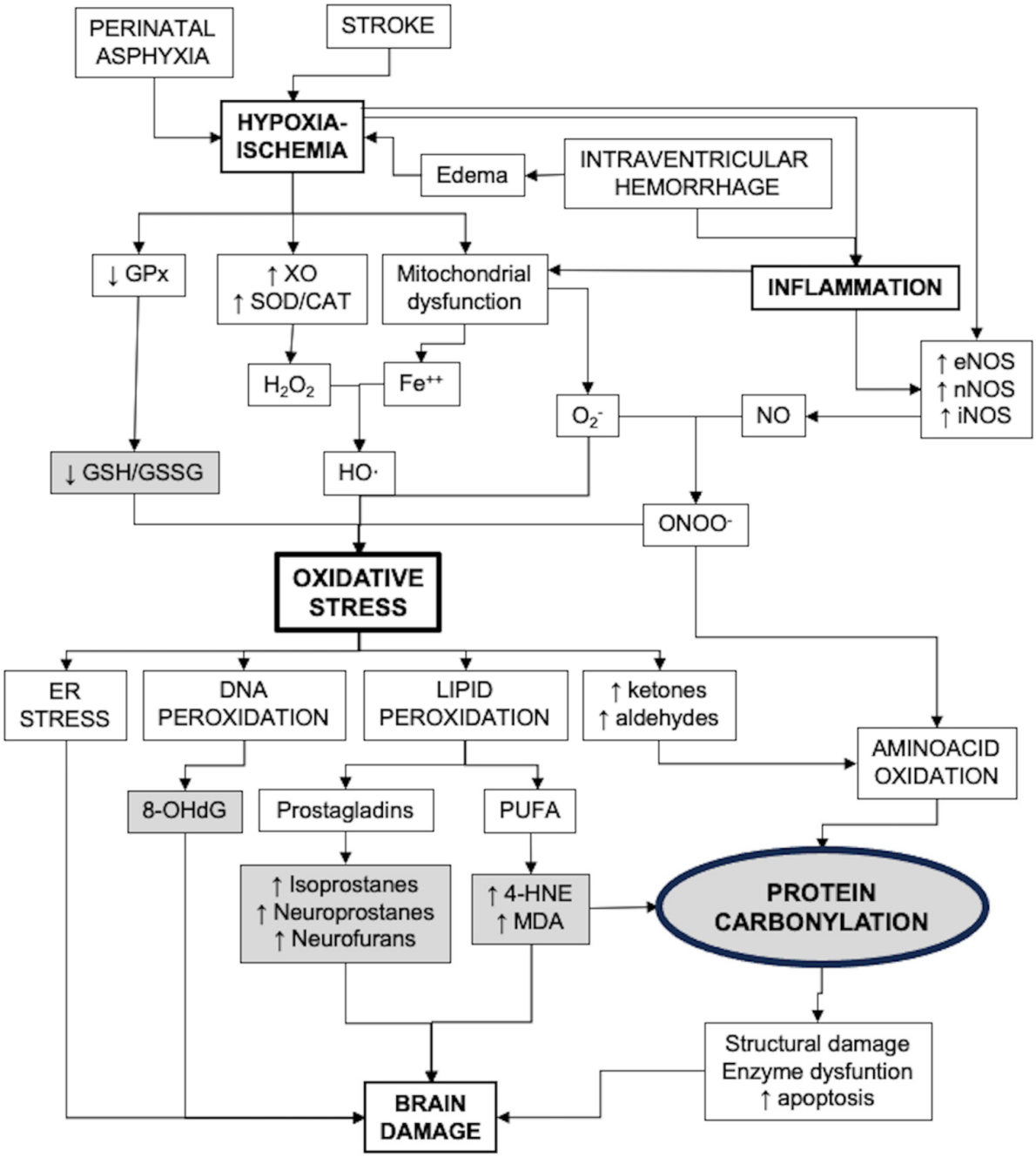

Figure 1. Factors involved in the development of oxidative stress after brain damage in newborns and the consequences. Strategic biomarkers are shown in gray shading. CAT: catalase; ER: endoplasmic reticulum; GPx: glutathione peroxidases; GSH: reduced glutathione; GSSG: glutathione bisulfide; MDA: malondialdehyde; 4-HNE: 4-hydroxynonenal; NOS: nitric oxide synthase; 8-OHdG: 8-hydroxy-deoxyguanosine; PUFA: poli-unsaturated fatty acids; SOD: superoxide dismutase; XO: xanthin oxidase.

2. Vulnerability of the Immature Brain to Oxidative Stress

The immature brain is notably susceptible to OS for multiple reasons. Firstly, the prenatal environment is characterized as a physiologically hypoxic environment, which results in underdeveloped antioxidant systems at birth, particularly evident in preterm newborns [5][6][7][10]. Moreover, the immature brain shows a heightened rate of oxygen consumption, a surplus of unsaturated fatty acids, and an elevated concentration of metals that catalyze reactive species formation [5][6][7][10]. Consequently, the disparity between reactive species production and antioxidant defenses is markedly accentuated in comparison to the mature brain [5][6][7][10]. Furthermore, the immature brain is highly predisposed to pathophysiological processes that augment OS. Notably, the greater density and heightened sensitivity of NMDA receptors elicit a more pronounced and deleterious response to extracellular glutamate [4][7][10]. Since glutamate reuptake enzymes are ATP-dependent, energy failure in an episode of hypoxia–ischemia leads to the extracellular accumulation of this excitotoxic neurotransmitter [4][7][10]. In fact, excitotoxicity, as an early and potent instigator, sets the stage for subsequent OS, giving rise to the concept of “excito-oxidative damage” [4]. Likewise, the immature brain is particularly susceptible to inflammatory processes, which further exacerbate OS [4][11].

A particular vulnerability of the immature brain is that, especially in the case of preterm newborns, the largest proportion of oligodendroglial cells, which will be responsible for the subsequent myelination process, are oligodendroglial precursors [12]. Among the cells constituting the oligodendroglial lineage, oligodendroglial precursors demonstrate the utmost sensitivity to OS [5][12][13]. This susceptibility arises chiefly due to their elevated iron content and heightened metabolic activity [5][12][13]. Notably, the heightened vulnerability of immature oligodendroglial cells to OS bestows upon the immature brain a distinct attribute. Unlike the adult brain, acute hypoxic–ischemic insults result in enduring myelination impairment, as observed in preclinical models of hypoxic–ischemic damage in neonatal rats [13].

Despite this vulnerability, the immature brain during the fetal period is exposed to multiple agents that can increase OS, such as environmental pollutants, infections or exposure to alcohol or other drugs [9][14][15][16]. Heavy metals, xeno-hormones, fertilizers, pesticides and herbicides are increasingly present in modern life and can reach the fetus through the mother, directly or indirectly generating an increase in OS [14]. Prenatal infections increase the inflammatory status in the fetus, either directly by contaminating the fetus itself or indirectly through the systemic inflammatory response that occurs in the mother [16]. As discussed later, inflammation is an important inducer of OS [7], including the impairment of mitochondrial function, which is an important cause of OS [16]. Among the multiple consequences of prenatal exposure to alcohol is the increase in OS, since alcohol downregulates antioxidant enzymes such as glutathione peroxidase and upregulates hydrogen peroxidase [15]. Additionally,

alcohol induces an inflammatory response in the immature brain through activation of microglia and alteration of astrocyte function [15]. Prenatal drug exposure may also result in increased OS [9]. Cocaine and methamphetamine may alter mitochondrial function and increase brain dopamine levels, leading to increased production of free radicals by auto-oxidation and metabolic oxidation by the action of monoamine oxidase [9]. Opioids increase lipid peroxidation and downregulate glutathione peroxidase to reduce brain glutathione concentration [9]. Nicotine, in addition to all these actions, is a strong and important source of nitric oxide (NO) [9], which, as will be discussed below, is also a damaging agent per se and because when produced in extraordinary quantities is one of the main agents causing protein carbonylation, which is one of the most serious consequences of OS [5][17].

In the present research researchers will focus on highly prevalent perinatal-acquired brain pathology in which oxidative stress plays an important role, such as diffuse or focal hypoxic–ischemic damage or the cerebral consequences of prematurity [1][2]. Furthermore, sick newborns are frequently exposed to episodes of hypoxia/hyperoxia, ischemia or infection, all of them leading to increased OS [5].

Thus, it is clear that OS plays a particularly prominent role in the process of brain damage in newborns [7].

3. Assessment of Oxidative Stress in Neonatal Brain Damage

Protein oxidation, due to its potential to disrupt the functionality of multiple proteins, plays a considerably detrimental role by perturbing the cellular redox balance and influencing the cell cycle, ultimately culminating in neuronal demise [8][17]. Among the characteristic consequences of OS, protein carbonylation emerges as an irrevocable modification, imparting notable damage [17][18]. Protein carbonylation involves the direct oxidation of lysine, arginine, proline, and threonine side chains, resulting in the formation of reactive ketones or aldehydes that subsequently react with 2,4-dinitrophenylhydrazine (DNPH) to yield hydrazones [18]. Carbonylated proteins are primarily degraded by the 20S proteasome, but after massive production carbonylated proteins, additional poorly understood mechanisms of decarbonylation are initiated, including thiol-dependent reduction and pyridoxamine scavenging [8]. When these systems are overwhelmed, carbonylated proteins accumulate in the cell, exerting cytotoxic effects [8]. Beyond mere structural and functional changes, protein carbonylation heightens cellular susceptibility to apoptotic triggers like calpains [17]. Consequently, protein carbonylation has garnered significance as an imperative marker of OS, not solely due to its structural and functional ramifications, but also due to its sensitivity and specificity [6][7][8][10].

A common difficulty in using protein carbonylation for research or diagnostic purposes is that, while LC-MS/MS is the established approach for detecting and quantifying oxidative markers, including carbonylated proteins, its extensive cost and requirement for substantial expertise constrain its widespread adoption [6]. To overcome this limitation, alternative methodologies have been developed to offer more accessible techniques in terms of both methodology and expenditure [6][10]. One of these methodologies is the Oxyblot technique, which was extensively used in the researchers' experiments (Oxyblot™, Merck Millipore, Merck KGaA, Darmstadt, Germany) [19][20][21][22]. Oxyblot is a commercially available Western blot method underpinned by anti-DNPH antibodies. It effectively detects carbonylated proteins, comparable in efficiency to other methods such as spectrophotometry or ELISA [17]

[23]. However, its distinctive merit lies in its user-friendly and cost-effective implementation [17][23]. The detection of protein carbonylation involves enhanced chemiluminescence, with quantification performed through densitometric analysis. The quantified data are subsequently normalized by considering the total protein loading, as obtained through the Red Ponceau technique in the sample [19][20][21][22].

Protein carbonylation can arise from the action of various oxidizing agents [18]. A major source is the reactive species OH·, a product of the Fenton reaction between H_2O_2 and ionic metals, in particular Fe^{2+}, that produces very toxic carbonyl derivatives of lysine, arginine and threonine [8]. Of special relevance are the peroxidation processes of polyunsaturated fatty acids, producing particularly toxic carbonyl species such as α,β-unsaturated aldehydes and ketoaldehydes, in particular the 4-HNE products, therefore playing a prominent role in the so-called secondary protein carbonylation processes [8][18]. Protein carbonylation can also result from non-enzymatic glycation, particularly in conditions with hyperglycemia, lysyl oxidation and polyphenol reactions [8]. Other major contributors to protein carbonylation are reactive nitrogen species (RNSs), resulting in protein nitrosylation [5][6][17]. RNSs primarily originate from the interaction of NO with other free radicals, engendering diverse reactive species, with peroxynitrite ($ONOO^-$) formation being the most detrimental consequence. $ONOO^-$ emerges from the union of NO and superoxide anions [5]. The synthesis of NO is facilitated by NO synthases (NOSs) [5][18]. Constitutive endothelial and neuronal NOSs (eNOS and nNOS, respectively) yield modest NO levels; conversely, inducible NOS (iNOS), once activated, triggers massive NO production through an irreversible process [5][24].

References

1. Molloy, E.J.; El-Dib, M.; Juul, S.E.; Benders, M.; Gonzalez, F.; Bearer, C.; Wu, Y.W.; Robertson, N.J.; Hurley, T.; Branagan, A.; et al. Neuroprotective Therapies in the NICU in Term Infants: Present and Future. Pediatr. Res. 2022, 93, 1819–1827.
2. Russ, J.B.; Simmons, R.; Glass, H.C. Neonatal Encephalopathy: Beyond Hypoxic-Ischemic Encephalopathy. Neoreviews 2021, 22, e148–e162.
3. Martínez-Orgado, J.; Villa, M.; Del Pozo, A. Cannabidiol for the Treatment of Neonatal Hypoxic-Ischemic Brain Injury. Front. Pharmacol. 2021, 11, 584533.
4. Johnston, M.V.; Fatemi, A.; Wilson, M.A.; Northington, F. Treatment Advances in Neonatal Neuroprotection and Neurointensive Care. Lancet Neurol. 2011, 10, 372–382.
5. Buonocore, G.; Groenendaal, F. Anti-Oxidant Strategies. Semin. Fetal Neonatal Med. 2007, 12, 287–295.
6. Martini, S.; Austin, T.; Aceti, A.; Faldella, G.; Corvaglia, L. Free Radicals and Neonatal Encephalopathy: Mechanisms of Injury, Biomarkers, and Antioxidant Treatment Perspectives. Pediatr. Res. 2020, 87, 823–833.

7. Torres-Cuevas, I.; Corral-Debrinski, M.; Gressens, P. Brain Oxidative Damage in Murine Models of Neonatal Hypoxia/Ischemia and Reoxygenation. Free Radic. Biol. Med. 2019, 142, 3–15.
8. Akagawa, M. Protein Carbonylation: Molecular Mechanisms, Biological Implications, and Analytical Approaches. Free Radic. Res. 2021, 55, 307–320.
9. Tsai, S.Y.A.; Bendriem, R.M.; Lee, C.T.D. The Cellular Basis of Fetal Endoplasmic Reticulum Stress and Oxidative Stress in Drug-Induced Neurodevelopmental Deficits. Neurobiol. Stress 2019, 10, 100145.
10. Tonni, G.; Leoncini, S.; Signorini, C.; Ciccoli, L.; De Felice, C. Pathology of Perinatal Brain Damage: Background and Oxidative Stress Markers. Arch. Gynecol. Obstet. 2014, 290, 13–20.
11. Hagberg, H.; Mallard, C.; Ferriero, D.M.; Vanucci, S.J.; Levison, S.W.; Vexler, Z.S.; Gressens, P. The Role of Inflammation in Perinatal Brain Injury. Nat. Rev. Neurol. 2015, 11, 192–208.
12. Volpe, J.J.; Kinney, H.C.; Jensen, F.E.; Rosenberg, P.A. Volpe The Developing Oligodendrocyte: Key Cellular Target in Brain Injury in the Premature Infant. Int. J. Dev. Neurosci. 2010, 29, 423–440.
13. Ceprián, M.; Vargas, C.; García-Toscano, L.; Penna, F.; Jiménez-Sánchez, L.; Achicallende, S.; Elezgarai, I.; Grandes, P.; Hind, W.; Ruth Pazos, M.; et al. Cannabidiol Administration Prevents Hypoxia-Ischemia-Induced Hypomyelination in Newborn Rats. Front. Pharmacol. 2019, 10, 131.
14. Al-Gubory, K.H. Environmental Pollutants and Lifestyle Factors Induce Oxidative Stress and Poor Prenatal Development. Reprod. Biomed. Online 2014, 29, 17–31.
15. Zhang, Y.; Wang, H.; Li, Y.; Peng, Y. A Review of Interventions against Fetal Alcohol Spectrum Disorder Targeting Oxidative Stress. Int. J. Dev. Neurosci. 2018, 71, 140–145.
16. Gyllenhammer, L.E.; Rasmussen, J.M.; Bertele, N.; Halbing, A.; Entringer, S.; Wadhwa, P.D.; Buss, C. Maternal Inflammation During Pregnancy and Offspring Brain Development: The Role of Mitochondria. Biol. Psychiatry Cogn. Neurosci. Neuroimaging 2022, 7, 498–509.
17. Oikawa, S.; Yamada, T.; Minohata, T.; Kobayashi, H.; Furukawa, A.; Tada-Oikawa, S.; Hiraku, Y.; Murata, M.; Kikuchi, M.; Yamashima, T. Proteomic Identification of Carbonylated Proteins in the Monkey Hippocampus after Ischemia-Reperfusion. Free Radic. Biol. Med. 2009, 46, 1472–1477.
18. Suzuki, Y.J.; Carini, M.; Butterfield, D.A. Protein Carbonylation. Antioxid. Redox Signal. 2010, 12, 323–325.
19. Lafuente, H.; Pazos, M.R.; Alvarez, A.; Mohammed, N.; Santos, M.; Arizti, M.; Alvarez, F.J.; Martinez-Orgado, J.A. Effects of Cannabidiol and Hypothermia on Short-Term Brain Damage in New-Born Piglets after Acute Hypoxia-Ischemia. Front. Neurosci. 2016, 10, 323.
20. Pazos, M.R.; Mohammed, N.; Lafuente, H.; Santos, M.; Martínez-Pinilla, E.; Moreno, E.; Valdizan, E.; Romero, J.; Pazos, A.; Franco, R.; et al. Mechanisms of Cannabidiol Neuroprotection in

Hypoxic-Ischemic Newborn Pigs: Role of 5HT(1A) and CB2 Receptors. Neuropharmacology 2013, 71, 282–291.

21. Del Pozo, A.; Villa, M.; Vargas, C.; Castejón, D.; Fernández-Valle, M.E.; Gutiérrez-Rodríguez, A.; Martínez-Orgado, J. Intraventricular Hemorrhage Induces Inflammatory Brain Damage with Blood–Brain Barrier Dysfunction in Immature Rats. Pediatr. Res. 2022, 93, 78–88.
22. Silva, L.; Vargas, C.; Prados, M.E.; del Pozo, A.; Villa, M.; Martínez, M.; Alvarez, L.; Muñoz, E.; Unciti-Broceta, J.D.; Martínez-Orgado, J. Neuroprotective Efficacy of Betulinic Acid Hydroxamate, a B55α/PP2A Activator, in Acute Hypoxia–Ischemia-Induced Brain Damage in Newborn Rats. Transl. Stroke Res. 2022, 14, 397–408.
23. Alomari, E.; Bruno, S.; Ronda, L.; Paredi, G.; Bettati, S.; Mozzarelli, A. Protein Carbonylation Detection Methods: A Comparison. Data Brief 2018, 19, 2215–2220.
24. Martínez-Orgado, J.; Fernández-López, D.; Moro, M.A.; Lizasoain, I. Nitric Oxide Synthase as a Target for the Prevention of Hypoxic-Ischemic Newborn Brain Damage. Curr. Enzym. Inhib. 2006, 2, 219–229.