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Review

Nuclear Factor Erythroid 2-Related Factor 2 as a Potential Therapeutic Target in Neonatal Hypoxic-Ischemic Encephalopathy

Jian-Xia Liu[1,†], Da Zheng[1,†], Ling Chen[1], Su Chen[1,*], Jia-Wei Min[1,*]

[1]Key Laboratory of Cognitive Science, Laboratory of Membrane Ion Channels and Medicine, College of Biomedical Engineering, South-Central Minzu University, 430079 Wuhan, Hubei, China

*Correspondence: ecloud.7@163.com (Su Chen); jiawei-min@hotmail.com (Jia-Wei Min)

†These authors contributed equally.

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Abstract

Hypoxic-ischemic encephalopathy (HIE) is a prominent cause of neonatal mortality and neurodevelopmental disorders; however, effective therapeutic interventions remain limited. During neonatal hypoxic-ischemic injury events, increased reactive oxygen species (ROS) production and decreased antioxidant levels lead to the induction of oxidative stress, which plays a pivotal role in the pathological process of neonatal HIE. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key endogenous antioxidant transcription factor that protects against oxidative stress by promoting the transcription of various antioxidant genes. It has been demonstrated that Nrf2 signaling pathway activation by different compounds may protect against neonatal HIE. This review outlines the role of oxidative stress in neonatal HIE and summarizes the impact of antioxidants on neonatal HIE via activation of the Nrf2 signaling pathway. In conclusion, Nrf2 signaling pathway potentially exerts antioxidant, anti-inflammatory, antiapoptotic and antiferroptotic effects, thereby emerging as a focal point for future neonatal HIE treatment strategies.

Keywords: neonatal brain; Nrf2; HIE; oxidative stress; inflammation; apoptosis; ferroptosis

1. Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE) is a neurological disorder induced by the disturbance of blood flow and oxygen supply to the brain [1]. HIE is considered a primary cause of disability and mortality in neonates [2,3]. The incidence of HIE is 1–8 per 1000 full-term infants in developed nations and approximately 26 per 1000 full-term infants in low-income and middle-income regions [4,5]. As a result of the hypoxic-ischemic insult in the brain, infants may have the acute clinical presentations of brain damage, such as abnormal fetal heart rate tracings, low Apgar scores or poor umbilical cord gases (pH $<$7.0 or base deficit $\geq$12 mmol/L) [6,7], and the long-term sensory/motor/cognitive disabilities, including visual and hearing impairment, cerebral palsy, cognitive and learning impairment, blindness, mental retardation, growth restriction, attention deficits and behavioral disabilities and HIE can also cause death [8–12]. To date, moderate hypothermia has been suggested as the only standard therapeutic approach for newborns with HIE. Moderate hypothermia must last for the therapeutic time window of 72 hours after the hypoxic-ischemic event [13]. Nevertheless, it can not provide complete neuroprotection against severe neonatal HIE [14].

One of the most broadly accepted pathophysiological mechanisms of neonatal HIE is oxidative stress [15]. Oxidative stress, which is involved in a variety of diseases, develops from overproduction of reactive oxygen species (ROS) and results in serious injury to cerebral tissue [16]. When ROS production exceeds the antioxidant capacity of molecules and enzymes, biological processes such as DNA and mitochondrial damage, protein carbonylation, lipid peroxidation and enzyme inactivation occur, resulting in inflammation, apoptosis, and ferroptosis and eventually brain damage [15,17–19]. Thus, it is of great importance to analyze the role of oxidative stress in neonatal HIE.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a crucial antioxidant transcription factor that can regulate several cytoprotective factors to reduce oxidative stress [20]. Under physiological conditions, Nrf2 remains at a low level via directed degradation by ubiquitylation [21]. When cells are exposed to oxidative stress, Nrf2 and Kelch-like ECH-associated protein 1 (Keap1) separate, and Nrf2 binds with the antioxidant response element (ARE) in the nucleus and then upregulates the levels of various antioxidant-encoding genes, such as heme oxygenase-1 (HO-1), glutathione (GSH), and nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase 1 [NQO1] [22]. By upregulating these antioxidants, Nrf2 is able to decrease ROS-mediated cellular injury and keep a dynamic redox balance [23]. A previous study revealed that genistein, which is a bioactive isoflavone phytoestrogen found in soybeans, exerted a neuroprotective effect against HIE in neonatal mice by reducing neuroinflammation and oxidative stress via the nuclear factor-kappa B (NF-κB) and Nrf2/HO-1 signaling pathways [24]. We also found that echinocystic acid (EA) mitigated hypoxic-ischemic brain damage (HIBD) in neonatal mice by ameliorating neuronal apoptosis and oxidative stress by activating the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/Nrf2 signaling pathway [25]. Thus, modulation of the Nrf2 signaling pathway may be a potential therapeutic strategy for neonatal HIE.

Here, we summarized the pathological mechanisms of oxidative stress in neonatal HIE and the modulation of the Nrf2 signaling pathway. Furthermore, we outlined a series of compounds that have been observed to provide neuroprotection in neonatal HIE models by reducing oxidative stress by activating the Nrf2 signaling pathway.

2. The Role of Oxidative Stress in Neonatal HIE

Following a hypoxic-ischemic event, large amounts of ROS are produced, which can lead to the induction of oxidative stress [15]. Oxidative stress is involved in a large number of pathophysiological processes, and in neonatal HIE, it induces inflammation, apoptosis and ferroptosis. In this section, we focus on the association between oxidative stress and neonatal HIE (Fig. 1).

2.1 Inflammation

Inflammation is a widespread cellular response associated with many neurological diseases [26]. Following hypoxic-ischemic injury, ROS take part in the activation of the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome [27], which can later increase the activity of caspase-1, pro-interleukin-18 (pro-IL-18) and pro-IL-1 [28]. Following the activation of caspase-1, proinflammatory cytokines such as interleukin-1β (IL-1β), IL-6, IL-1, IL-18, tumor necrosis factor-α (TNF-α) and transforming growth factor-beta (TGF-β) are released into the blood circulation, therefore boosting inflammatory responses and inducing neuronal injury [29–32]. Moreover, disrupted cells release damage-associated molecular patterns (DAMPs), which bind to Toll-like receptors (TLRs) and activate the NF-κB signaling pathway to enhance inflammatory responses [15,33,34]. The available evidence suggested that licochalcone A (LCA), which is the main phenolic component of licorice, could provide neuroprotection against oxygen-glucose deprivation/reoxygenation (OGD/R)-induced neuronal damage by attenuating ROS-induced inflammation [35]. Similarly, Cheng *et al.* [36] found that treatment with gelsemine inhibited inflammatory responses by ameliorating oxidative stress and alleviating memory impairments and spatial learning in neonatal HIE models. Therefore, the ROS-induced inflammatory response is an important part of the pathophysiology of neonatal HIE.

2.2 Apoptosis

Apoptosis is a programmed cell death mode in mammals. The release of excessive ROS from mitochondria triggers the release of hydrolytic enzymes from lysosomes, which activates the B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax), eventually increasing the permeability of the mitochondrial membrane and resulting in the release of cytochrome c (Cytc) from the mitochondria [15]. Once released into the cytoplasm, Cytc forms an apoptotic complex with the caspase-9 zymogen. Subsequently, activated caspase-9 initiates the activation of caspase-3, which ulti-

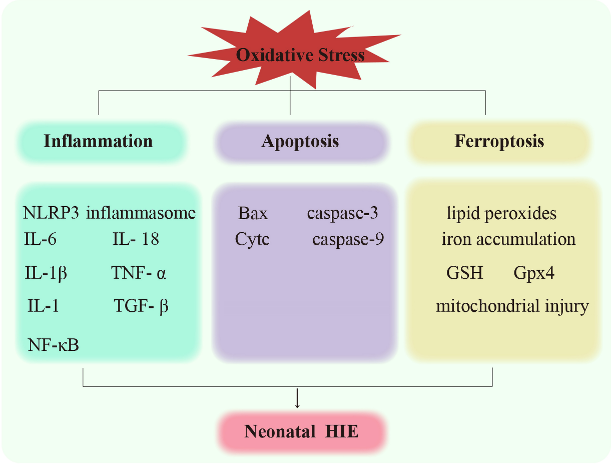

Fig. 1. Oxidative stress and neonatal HIE. Following a hypoxic-ischemic event, large amounts of ROS are produced, which can lead to the induction of oxidative stress. Additionally, ROS take part in the activation of the NLRP3 inflammasome, which can later increase the activity of caspase-1, pro-IL-1 and pro-IL-18, and then proinflammatory cytokines (e.g., TNF-α, IL-6, IL-1, IL-18, TGF-β and IL-1β) are released into the blood circulation, thereby stimulating intracellular inflammatory responses. Moreover, excessive ROS induce the activation of NF-κB to enhance the inflammatory response. Similarly, overproduced ROS can trigger apoptosis. The proapoptotic protein Bax changes mitochondrial membrane permeability and stability, Cytc is released, and Cytc forms an apoptotic complex with the caspase-9 zymogen. Subsequently, activated caspase-9 initiates the activation of caspase-3, which activates the cleavage of DNA substrates. Furthermore, hypoxic-ischemic conditions result in the formation of ROS, which increases lipid peroxidation, promotes iron accumulation, reduced GSH and Gpx4 activity and induces mitochondrial injury, ultimately leading to the development of ferroptosis. Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Cytc, cytochrome c; GSH, glutathione; Gpx4, glutathione peroxidase 4; IL-1, interleukin-1; IL-6, interleukin-6; IL-18, interleukin-18; IL-1β, interleukin-1β; NF-κB, nuclear factor-kappa B; NLRP3, the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; pro-IL-18, pro-interleukin-18; ROS, reactive oxygen species; TNF-α, tumor necrosis factor α; TGF-β, transforming growth factor-beta; HIE, hypoxic-ischemic encephalopathy.

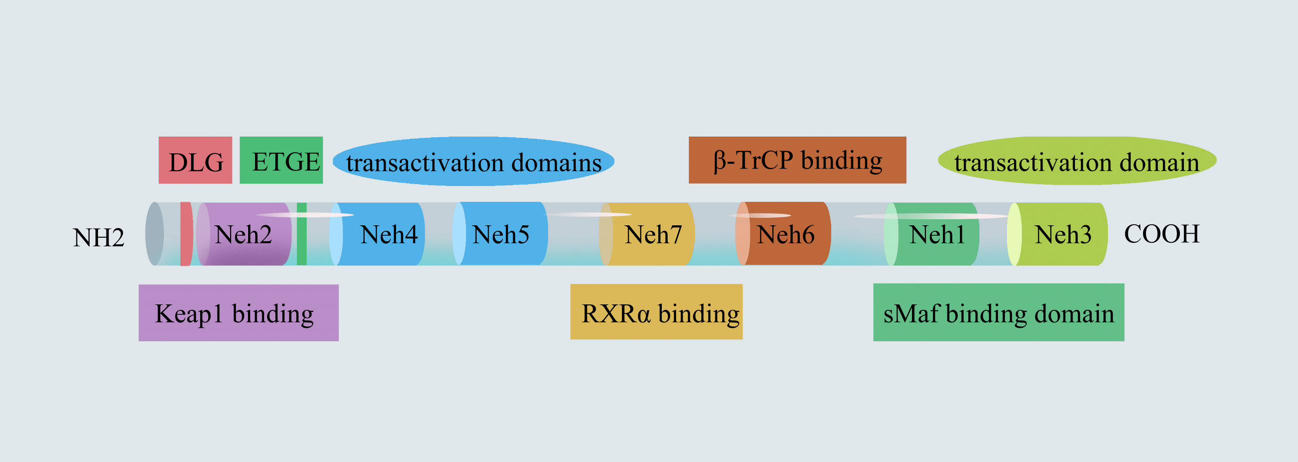

Fig. 2. Region structures of Nrf2. The Nrf2 protein includes 7 Neh regions: Neh1-Neh7. The Neh2 region interacts with Keap1 through the DLG and ETGE motifs. The Neh4, Neh5 and Neh3 regions are vital for transactivation. The Neh7 region binds to RXRα, and Neh6 interacts with β-TrCP to regulate Nrf2 stability. The Neh1 domain heterodimerizes with sMaf. DLG, Asp-Leu-Gly; ETGE, Glu-Thr-Gly-Glu; Neh1-7, Nrf2-ECH homology 1-7; β-TrCP, β-transducin repeat-containing protein; RXRα, retinoid X receptor-alpha; sMaf, small musculoaponeurotic fibrosarcoma; Keap1, Kelch-like ECH-associated protein 1.

mately cleaves the DNA fragment of the substrate and leads to cell death [37]. Hypoxic-ischemic conditions lead to inadequate provision of nutrients and oxygen to cells, resulting in a considerable amount of neuronal apoptosis [38–40]. A previous study showed that lycopene (Lyc), which is a carotenoid compound isolated from tomatoes, reduced ROS-induced apoptosis in both *in vivo* and *in vitro* experiments [41]. Likewise, Chen *et al.* [42] indicated that myricetin exerted a neuroprotective effect on the neonatal rat brain by mitigating ROS-induced neuronal apoptosis and ameliorated long-term neurological prognosis and infarct volume in neonatal HIE models. Thus, ROS-related apoptosis plays an important role in brain damage associated with neonatal HIE.

2.3 Ferroptosis

Ferroptosis is a programmed cell death mode induced by the anomalous metabolism of iron, lipids and GSH and characterized by iron-regulated lipid peroxidative accumulation [43]. Hypoxic-ischemic conditions lead to the formation of ROS and lipid peroxidation, iron accumulation, reduced GSH and glutathione peroxidase 4 (Gpx4) activity, and mitochondrial injury, ultimately leading to the development of ferroptosis [44–47]. Ferroptosis results in the accumulation of iron-regulated ROS, reduction in cellular antioxidant capacity, and subsequent cell death, which can increase central nervous system damage [43,48]. Recent research has shown that exogenous melatonin (Mel) can alleviate hypoxic-ischemic brain injury by reducing ROS-induced ferroptosis and exert obvious neuroprotective effects on neonatal HIE models [49]. Furthermore, Cai *et al.* [50] found that treatment with vitamin D (VD) efficiently reduced ferroptosis by suppressing oxidative stress and provided neuroprotection in neonatal HIE models. Hence, ROS-induced ferroptosis is considered to be an important pathophysiological factor of neonatal HIE.

3. The Role of Nrf2 in Combating Oxidative Stress

The Nrf2 protein is a member of the Cap-n-Collar family of transcription factors and has 7 functional domains named Nrf2-ECH homology 1-7 (Neh1-7) [51–53] (Fig. 2). The Neh1 domain modulates small musculoaponeurotic fibrosarcoma (sMaf) proteins and DNA binding and is responsible for promoting Nrf2 nuclear translocation [23]. The Neh2 region interacts with Keap1 via the high-affinity Glu-Thr-Gly-Glu (ETGE) and low-affinity Asp-Leu-Gly (DLG) of the degenerate motif of the Neh2 structure [54]. Keap1 is a cysteine-rich inhibitor of Nrf2 that regulates the stability and ubiquitination of Nrf2 [55]. The interactions of Nrf2 with other coactivators are mediated by Neh3, Neh4, and Neh5 [56]. Neh6 is a negatively regulated structural domain that leads to the ubiquitination of Nrf2 by binding to the β-transducin repeat-containing protein (β-TrCP) [57,58]. The structural domain of Neh7 obstructs the Nrf2/ARE signaling pathway by promoting the binding of Nrf2 to retinoid X receptor-alpha (RXRα) [23,58,59].

Nrf2 is one of the vital regulators of endogenous antioxidant production and plays an important role in improving cerebral injury [60,61]. Under physiological conditions, Nrf2 remains at a low level due to its direct degradation through ubiquitylation [21]. Following a hypoxic-ischemic event, Nrf2 is separated from Keap1 and subsequently migrates from the cytoplasm into the nucleus [62]. Then, Nrf2 can heterodimerize with the transcriptional regulator sMaf [63,64], bind to ARE [65–69], and upregulate the expres-

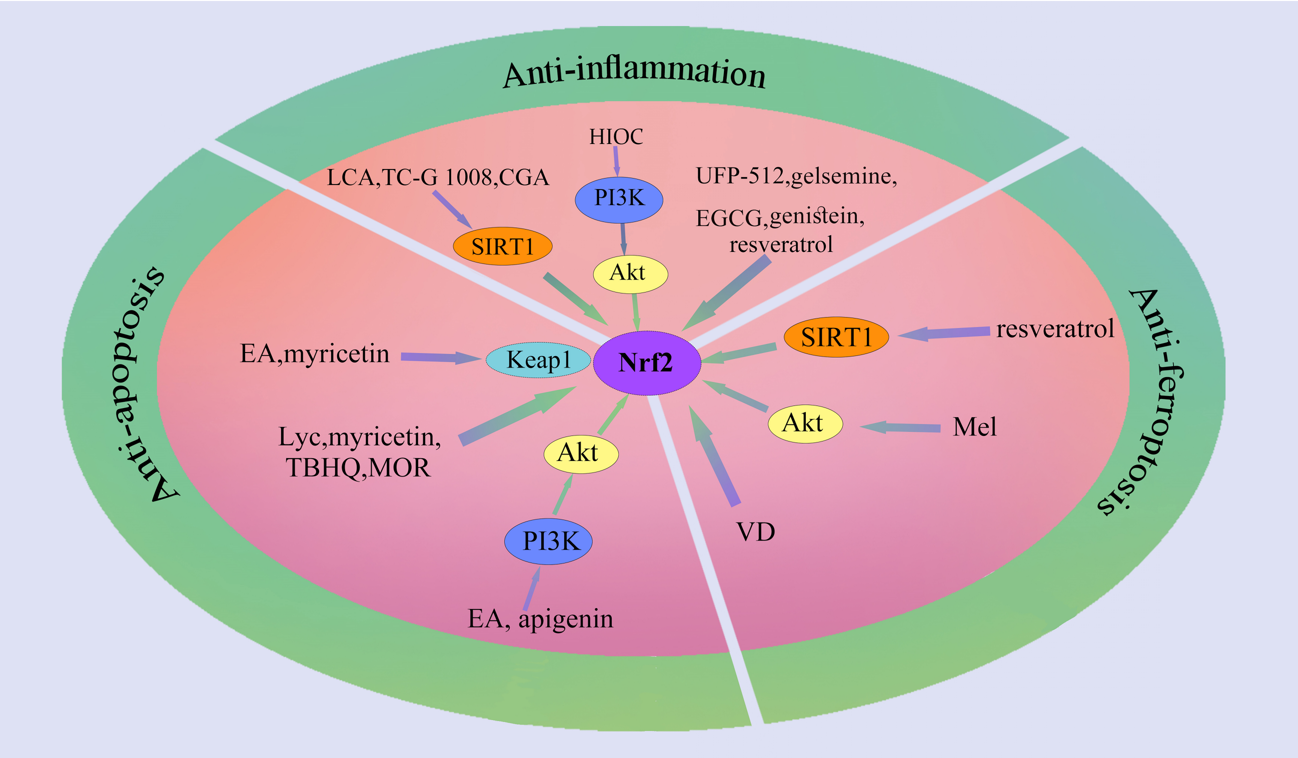

Fig. 3. Compounds that activated Nrf2 in neonatal HIE. Neuroprotective compounds showed anti-inflammatory, antiapoptotic and anti-ferroptotic effects on neonatal HIE via activation of the Nrf2 signaling pathway or Nrf2-related pathway. Akt, protein kinase B; CGA, chlorogenic acid; EA, echinocystic acid; EGCG, epigallocatechin gallate; HIOC, N-[2-(5-hydroxy-1H-indol-3-yl) ethyl]-2-oxopiperidine-3-carboxamide; Keap1, Kelch-like ECH-associated protein 1; LCA, licochalcone A; Lyc, lycopene; Mel, melatonin; MOR, morroniside; Nrf2, nuclear factor erythroid 2-related factor 2; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; SFN, sulforaphane; SIRT1, sirtuin 1; TBHQ, tertiary-butylhydroquinone; VD, vitamin D; UFP-512, H-Dmt-Tic-NH-CH(CH2–COOH)-Bid; TC-G 1008, G-protein-coupled receptor 39-C3.

sion of various enzymes involved in antioxidant protection, such as superoxide dismutase (SOD), glutathione peroxidase (Gpx) and catalase (CAT) [70]. By upregulating these antioxidant enzymes, Nrf2 can reduce ROS production and inhibit oxidative stress-induced cellular damage and ultimately keep a dynamic redox balance [70–75]. Furthermore, Zhang *et al*. [76] found that Nrf2 knockout aggravated brain infarct after neonatal hypoxic-ischemic (HI) injury. Therefore, the Nrf2 signaling pathway plays a crucial part in the regulation of oxidative stress after neonatal HIE.

4. Effects of Nrf2 Modulators on Neonatal HIE

Different compounds have been shown to exert great neuroprotective effects on neonatal HIE by ameliorating oxidative damage through the activation of Nrf2 (Table 1, Ref. [24,25,35,36,41,42,44,49,50,77–86]). These compounds can be divided into three types: against ROS-induced inflammation, against ROS-induced apoptosis, and against ROS-induced ferroptosis (Fig. 3). The terms Nrf2, oxidative stress and neonatal brain were searched in PubMed to find relevant studies.

4.1 Compounds Targeting Oxidative Stress and Inflammation

Following HI conditions, the inflammatory response is triggered by ROS, and the activation of Nrf2 can suppress inflammation via ROS elimination [86,87]. Next, we summarized some compounds that provided neuroprotection in neonatal HIE models by inhibiting ROS-induced inflammation by activating Nrf2-related pathways or the Nrf2 signaling pathway.

Licochalcone A (LCA) is the main phenolic component of licorice. A recent study has found that LCA administration counteracts OGD/R-induced downregulation of sirtuin 1 (SIRT1)/Nrf2 and exerts beneficial effects on rat primary cortical neurons by ameliorating ROS-induced inflammation. LCA upregulated the expression of SOD, Nrf2, SIRT1, HO-1 and Gpx and decreased ROS, lactate dehydrogenase (LDH), malondialdehyde (MDA), TNF-α and IL-6 levels [35]. Similarly, Xie *et al*. [77] found that G-protein-coupled receptor 39-C3 (TC-G) 1008 (15 mg/kg), which is an agonist of G-protein-coupled receptor 39 (GPR39), intranasally attenuated ROS-induced neuroinflammation in neonatal HIBD model rats through the SIR-

Table 1. Various compounds attenuate neonatal HIE via the activation of Nrf2.

Compounds	Model	Dose and mode of administration of drugs	Efficacy	Results	Reference
LCA	OGD/R (primary cortical neurons)	*In vitro*: 0, 10, 20 and 40 μM during OGD/R (28 h)	SOD ↑, Gpx ↑, Nrf2 ↑, HO-1 ↑, LDH ↓, ROS ↓, MDA ↓, TNF-α ↓, IL-6 ↓	Reduces oxidative stress and inflammatory response	[35]
TC-G 1008	HIBD model (SD)	*In vivo*: 15 mg/kg intranasally at 1, 25, 49, and 73 h after HIBD	Nrf2 ↑, IL-1β ↓, IL-6 ↓, TNF-α ↓	Reduces oxidative stress and inflammatory response	[77]
HIOC	HIBD model (SD) and OGD/R model (primary cortical neurons)	*In vivo*: 10 mg/kg, intraperitoneally at 12, 24 and 48 h after HIBD; *In vitro*: 100, 250, 500 and 750 nM, pretreatment	Nrf2 ↑, HO-1 ↑, Bcl-2 ↑, Bax ↓, TNF-α ↓, NLRP3 inflammasome ↓, caspase1 p20 ↓, IL-1β ↓	Inhibits oxidative stress, inflammation and apoptosis	[78]
Resveratrol	HIBD model (SD)	*In vivo*: 20 and 40 mg/kg intraperitoneally after 7 days of pretreatment	Nrf2 ↑, HO-1 ↑, Gpx ↑, SOD ↑, CAT ↑, MDA ↓, IL-6 ↓, IL-1β ↓, TNF-α ↓	Reduces oxidative stress and inflammatory response	[79]
UFP-512	HIBD model (SD)	*In vivo*: 5 mg/kg intraperitoneally before HIBD	Nrf2 ↑, HO-1 ↑, NQO1 ↑, IL-10 ↑, TNF-α ↓, IL-6 ↓, ICAM-1 ↓	Reduces oxidative stress and inflammatory response	[80]
Gelsemine	HIBD model (C57BL/6) and OGD/R model (BV2)	*In vivo*: 10 μg/kg intraperitoneally at 20 min before HIBD; *In vitro*: 100 nM for 3h before OGD/R	SOD ↑, HO-1 ↑, Nrf2 ↑, MDA ↓, TNF-α ↓, IL-1β ↓, IL-6 ↓, LDH ↓, ROS ↓	Reduces oxidative stress and inflammatory response	[36]
Myricetin	HIBD model (SD) and OGD/R model (PC12 cell)	*In vivo*: 25 mg/kg gavage starting 1 h after HI; *In vitro*: 200 μM, pretreatment for 2 h	Nrf2 ↑, HO-1 ↑, NQO1 ↑, Bcl-2 ↑, MDA ↓, ROS ↓, cleaved-caspase-3 ↓, Bax ↓, Keap1 ↓	Inhibits oxidative stress and apoptosis	[42]
TBHQ	HIBD model (SD)	*In vivo*: 20 mg/kg intraperitoneally after 1 h of HI and repeated once daily for 7 consecutive days	Nrf2 ↑, NQO1 ↑, SOD2 ↑, HO-1 ↑, IL-10 ↑, MDA ↓, ROS ↓, IL-1β ↓, ICAM-1 ↓, caspase3 ↓, caspase9 ↓	Inhibits apoptosis, inflammation and oxidative stress	[81]
MOR	OGD/R model (HT-22 cells)	*In vitro*: 5, 10 and 20 μM for 2 h and then exposed to OGD/R	SOD ↑, Gpx ↑, Bcl-2 ↑, Nrf2 ↑, HO-1 ↑, ROS ↓, MDA ↓, Bax ↓, caspase-3 ↓, caspase9 ↓	Inhibits oxidative stress, inflammation and apoptosis	[82]
EGCG	OGD/R model (BV2)	*In vitro*: 50, 100, 150, 200 and 250 μM, pretreatment for 1 h	HO-1 ↑, Nrf2 ↑, IL-6 ↓, iNOS ↓, COX-2 ↓, ROS ↓, HIF-1α ↓, caspase-3 ↓	Inhibits oxidative stress, inflammation and apoptosis	[83]
CGA	HIBD model (SD) and OGD/R model (primary cortical neurons)	*In vivo*: 150, 300 and 600 mg/kg intraperitoneally for 3 days after HIBD; *In vitro*: 100, 200 and 300 μM after OGD	CAT ↑, HO-1 ↑, Nrf2 ↑, SOD2/MnSOD ↑, MDA ↓, IL-1β ↓, iNOS ↓, TNF-α ↓	Inhibits oxidative stress, inflammation and apoptosis	[84]
Genistein	HIBD model (C57BL/6) and OGD/R model (primary cortical neurons)	*In vivo*: 10 mg/kg, intraperitoneally once daily for 3 consecutive days before the operation; *In vitro*: 5, 7.5, 10, 12.5 and 15 μM for 24 h before OGD/R and during OGD/R	Bcl-2 ↑, GSH ↑, Nrf2 ↑, HO-1 ↑, TNF-α ↓, IL-1β ↓, IL-6 ↓, Bax ↓, MDA ↓, LDH ↓, cleaved-caspase-3 ↓	Inhibits oxidative stress, inflammation and apoptosis	[24]

Table 1. Continued.

Compounds	Model	Dose and mode of administration of drugs	Efficacy	Results	Reference
Lyc	HIBD model (SD) and OGD/R model (primary cortical neurons)	*In vivo*: 5, 10 and 20 mg/kg intragastrically after HIBD; *In vitro*: 2.5, 5 and 10 µM after OGD insult	Bcl-2 ↑, HO-1 ↑, Nrf2 ↑, IL-1β ↓, IL-6 ↓, TNF-α ↓, Bax ↓, IL-18 ↓, cleaved-caspase-3 ↓	Inhibits apoptosis, inflammation and oxidative stress	[41]
EA	HIBD model (C57BL/6) and OGD/R model (primary cortical neurons)	*In vivo*: 50, 75 and 100 mg /kg intraperitoneally after HIBD; *In vitro*: 5, 10, 15 and 20 µM during OGD/R	Nrf2 ↑, NQO1 ↑, HO-1 ↑, Bcl-2 ↑, GSH ↑, Bax ↓, MDA ↓, ROS ↓, Keap1 ↓, cleaved caspase-3 ↓	Inhibits apoptosis and oxidative stress	[25]
Apigenin	HIBD model (SD)	*In vivo*: 10, 20 and 40 mg/kg intragastrically after HIBD	Nrf2 ↑, HO-1 ↑, Bcl-2 ↑, Mcl-1 ↑, Bax ↓, cleaved-caspase-3 ↓	Inhibits oxidative stress, inflammation and apoptosis	[85]
VD	HIBD model (SD) and OGD/R model (SH-SY5Y cells)	*In vivo*: 0.1 µg/kg intraperitoneally for 2 weeks; *In vitro*: 20 ng/mL prior to OGD	SOD ↑, GSH ↑, Nrf2 ↑, HO-1 ↑, Gpx4 ↑, MDA ↓, ROS ↓, IL-6 ↓, IL-1β ↓, TNF-α ↓	Suppresses ferroptosis	[50]
Mel	HIBD model (SD)	*In vivo*: 10 mg/kg intraperitoneally after HIBD	GSH ↑, Gpx4 ↑, Nrf2 ↑, 4-HNE ↓	Suppresses ferroptosis	[49]
Resveratrol	HIBD model (SD)	*In vivo*: 25 µg/3 µL intracerebroventricularly for 30 min before HIBD	Gpx4 ↑, Nrf2 ↑, MDA ↓, iron ↓	Suppresses ferroptosis	[44]
SFN	HIBD model (SD)	*In vivo*: 5 mg/kg intraperitoneally for 30 min before HIBD	Nrf2 ↑, HO-1 ↑, MDA ↓, 8OH-dG ↓, caspase-3↓	Inhibits apoptosis, inflammation and oxidative stress	[86]

Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; CAT, catalase; CGA, chlorogenic acid; COX-2, cyclooxygenase-2; EA, echinocystic acid; EGCG, epigallocatechin gallate; GSH, glutathione; Gpx, glutathione peroxidase; Gpx4, glutathione peroxidase 4; h, hour; HI, hypoxic-ischemic; HIBD, hypoxic-ischemic brain damage; HIF-1α, hypoxia-inducible factor-1α; HO-1, heme oxygenase-1; HIOC, N-[2-(5-hydroxy-1H-indol-3-yl) ethyl]-2-oxopiperidine-3-carboxamide; IL-1β, interleukin-1β; iNOS, inducible nitric oxide synthase; ICAM-1, intercellular cell adhesion molecule-1; IL-18, interleukin-18; IL-10, interleukin-10; IL-6, interleukin 6; Keap1, kelch-like ECH-associated protein 1; LDH, lactate dehydrogenase; LCA, licochalcone A; Lyc, lycopene; Mcl-1, myeloid cell leukemia-1; Mel, melatonin; MDA, malondialdehyde; MOR, morroniside; MnSOD, manganese-superoxide dismutase; Nrf2, nuclear factor erythroid 2-related factor 2; NADPH, nicotinamide adenine dinucleotide phosphate; NQO1, NAD(P)H quinone oxidoreductase 1; NLRP3, the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; OGD/R, oxygen-glucose deprivation/reoxygenation; ROS, reactive oxygen species; SD, Sprague-Dawley; SFN, sulforaphane; SOD, superoxide dismutase; TNF-α, tumor necrosis factor α; TBHQ, tertiary-butylhydroquinone; VD, vitamin D; 4-HNE, 4-hydroxynonenal; 8OH-dG, 8-hydroxy-2′-deoxyguanosine; UFP-512, H-Dmt-Tic-NH-CH(CH2–COOH)-Bid; TC-G 1008, G-protein-coupled receptor 39-C3.

-T1/peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α)/Nrf2 signaling pathway. TC-G 1008 administration significantly upregulated the levels of SIRT1, PGC-1α and Nrf2 and decreased the expression of IL-6, IL-1β, and TNF-α. TC-G 1008 also alleviated neurological deficits and the infarcted area. Likewise, Zheng *et al.* [84] confirmed that chlorogenic acid (CGA), which is a phenolic compound, increased the nuclear transfer of Nrf2 and the expression levels of SIRT1, HO-1, CAT and SOD2/manganese-superoxide dismutase (MnSOD) in a neonatal HIBD model. CGA exerted a protective role by inhibiting oxidative stress and the inflammatory response and reducing the levels of MDA, inducible nitric oxide synthase (iNOS), IL-1β, and TNF-α. CGA also alleviated structural tissue damage and brain edema after HI. In addition, CGA treatment improved cognitive ability and long-term spatial memory in neonatal HIBD rats. Furthermore, current research has indicated that N-[2-(5-hydroxy-1H-indol-3-yl) ethyl]-2-oxopiperidine-3-carboxamide (HIOC), which is a derivative of N-acetylserotonin, increases the nuclear translocation of Nrf2, the protein expression levels of phosphorylated PI3K (p-PI3K) and phosphorylated Akt (p-Akt), and the levels of Bcl-2 and HO-1 in both *in vivo* and *in vitro* models. HIOC administration showed a neuroprotective effect against oxidative stress injury and the inflammatory response, inhibited the activation of the NLRP3 inflammasome and downregulated the expression of Bax, TNF-α, caspase1 p20 and IL-1β. Additionally, HIOC also suppressed cerebral edema, cerebral infarction volume and neuronal apoptosis [78].

A previous study has shown that H-Dmt-Tic-NH-CH(CH2–COOH)-Bid (UFP-512), which is a potent δ-opioid receptor (DOR) agonist that is widely dispersed in the brain, plays a neuroprotective function in neonatal HIBD rat by inhibiting oxidative stress and the inflammatory response via the Nrf2/HO-1/ NAD(P)H quinone oxidoreductase 1 (NQO-1) signaling pathway. The activation of DOR substantially restrained the production of HI-induced TNF-α, intercellular adhesion molecule-1 (ICAM-1) and IL-6 and significantly increased the levels of IL-10, Nrf2, HO-1 and NQO-1 [80]. Moreover, gelsemine, an alkaloid derived from *Gelsemium elegans*, promoted the nuclear translocation of Nrf2 and attenuated oxidative stress and the inflammatory response in both *in vivo* and *in vitro* models after HI. Gelsemine also alleviated memory impairments and spatial learning in neonatal mice after HI [36]. Likewise, Kim *et al.* [83] reported that epigallocatechin gallate (EGCG), which is the main polyphenolic compound, has effective antioxidant capabilities and suppressed hypoxia-mediated oxidative stress and inflammation in $CoCl_2$-treated BV2 microglia by activating Nrf2/HO-1 signaling pathway. EGCG upregulated nuclear Nrf2 expression and downregulated cytosolic Nrf2 expression. EGCG also increased HO-1 levels and decreased caspase-3 activation, as well as the expression levels of iNOS, cyclooxygenase (COX)-2, ROS, hypoxia-inducible factor-1α (HIF-1α) and IL-6. Moreover, EGCG inhibited chronic inflammation and microglial activation. Similarly, our study found that genistein, which is a bioactive isoflavone phytoestrogen found in soybeans, can increase the expression of nuclear Nrf2 and cytosolic HO-1 proteins and mitigate the ROS-induced inflammatory response in both *in vivo* and *in vitro* models after HI. Genistein also upregulated the levels of Bcl-2 and GSH, downregulated the expression of Bax and cleaved caspase-3, and decreased the expression levels of LDH, IL-1β, MDA, IL-6 and TNF-α in neonatal HIBD mice. Administration of genistein effectively attenuated brain atrophy, cerebral infarction, neuronal damage and apoptosis [24]. Likewise, Gao *et al.* [79] found that resveratrol, which is widely present in polyphenolic compounds, ameliorated oxidative stress injury and the inflammatory response in HI-induced neonatal rats by positively modulating the Nrf2/HO-1 signaling pathway. They indicated that treatment with resveratrol upregulated the expression of Gpx, CAT & SOD and the protein expression levels of Nrf2 (nuclear fraction) and HO-1 (cytosolic fraction) and decreased the production of MDA, IL-1β, IL-6 and TNF-α in the cerebral cortex homogenate.

Collectively, these results indicate that these compounds may exert neuroprotective effects in neonatal HIE models by activating Nrf2-related pathways or the Nrf2 signaling pathway to relieve ROS-induced inflammation.

4.2 Compounds Targeting Oxidative Stress and Apoptosis

After hypoxic-ischemic injury, the accumulation of excess ROS can lead to apoptosis, and apoptosis can be inhibited by activating Nrf2 [88–90]. Subsequently, we outlined several compounds that provided neuroprotection by ameliorating ROS-induced apoptosis by activating Nrf2-related pathways or the Nrf2 signaling pathway in neonatal HIE models.

Our previous study has confirmed that echinocystic acid (EA), which is an herbal pentacyclic triterpene, increases the ratios of p-PI3K/PI3K and p-Akt/Akt and the expression level of Nrf2 and exerts a neuroprotective effect against hypoxia-induced ischemic brain damage and ROS-induced neural apoptosis in both *in vivo* and *in vitro* models. EA administration also upregulated the expression of Bcl-2, GSH, NQO1 and HO-1 and downregulated the expression of Bax, MDA, ROS, Keap1 and cleaved caspase-3. Moreover, EA could effectively alleviate neuronal injury and long-term neurobehavioral deficits and decrease cerebral infarction and brain atrophy after HI [25]. Similarly, apigenin is widely found in chamomile tea, vegetables and celery and has a variety of biological properties, such as antioxidant, anti-inflammatory and antiapoptotic properties. Treatment with apigenin provided neuroprotection in neonatal HIBD rats by inhibiting ROS-induced apoptosis through the activation of the PI3K/Akt/Nrf2 signaling pathway. Apigenin especially decreased the expression of

Bax and cleaved caspase-3 and upregulated the expression of Bcl-2, HO-1, Nrf2 and myeloid cell leukemia-1 (Mcl-1). Moreover, apigenin administration also markedly decreased cerebral edema and inflammation and improved structural tissue damage following HI [85].

Lycopene (Lyc) is a carotenoid compound isolated from tomatoes. Fu *et al.* [41] found that treatment with Lyc was able to significantly upregulate the protein levels of Nrf2 and HO-1 and ameliorated ROS-mediated apoptosis and hypoxia-induced ischemic brain damage in both *in vivo* and *in vitro* HI models. Furthermore, Chen *et al.* [42] demonstrated that myricetin, which is a naturally occurring flavonol compound, could attenuate neonatal HI brain injury by alleviating ROS-induced apoptosis through activating the Nrf2 signaling pathway in both *in vivo* and *in vitro* HI models. Myricetin could increase the expression levels of NQO-1, Nrf2, Bcl-2 and HO-1 and decrease the expression of MDA, ROS, Bax, Keap1 and cleaved caspase-3. In addition, myricetin also effectively reduced glial activation and brain infarction volume in HI injury. Similarly, Zhang *et al.* [81] indicated that tertiary-butylhydroquinone (TBHQ), which is an Nrf2 activator, promoted the nuclear transcription of Nrf2 and suppressed oxidative stress and apoptosis in neonatal HIBD rats. TBHQ increased the expression of Nrf2, HO-1, NQO1, SOD2 and IL-10 and downregulated the levels of ROS, MDA, caspase-3, caspase-9, ICAM-1 and IL-1β. TBHQ also reduced neuronal damage and infarct size and promoted motor coordination, neurological reflexes while alleviating memory deficits in neonatal HIBD mice. Additionally, the silencing of Nrf2 inhibited these beneficial effects. Likewise, Zhang *et al.* [82] demonstrated that morroniside (MOR), which is a natural secoiridoid glycoside derived from Cornusfficinalis that has antioxidative and antiapoptotic properties, ameliorated ROS-induced apoptosis and inflammation in OGD/R-induced HT-22 cells by activating the Nrf2/HO-1 signaling pathway. MOR upregulated the nuclear expression of Nrf2 and the activity of SOD, Gpx, Bcl-2 and HO-1 and obviously decreased the expression of ROS, MDA, Bax, cleaved caspase-3 and cleaved caspase-9, suggesting that the neuroprotection of MOR is mediated by the Nrf2/HO-1 signaling pathway. Similarly, a previous study has shown that sulforaphane (SFN), which is an isothiocyanate compound found in cruciferous vegetables, exerts a neuroprotective effect against ROS-induced apoptosis and inflammation by activating the Nrf2/HO-1 signaling pathway in neonatal HIBD rats. SFN also reduces the expression of MDA, 8-hydroxy-2′-deoxyguanosine (8OHdG) and caspase-3. Furthermore, SFN treatment alleviates infarct ratio [86].

In general, the instances summarized above indicate that these compounds could ameliorate ROS-induced apoptosis and hold great promise for the treatment of neonatal HIE by activating the Nrf2-related pathway or Nrf2 signaling pathway.

4.3 Compounds Targeting Oxidative Stress and Ferroptosis

After the occurrence of neonatal HIE, the accumulation of excess ROS could result in ferroptosis. However, activation of Nrf2 can ameliorate ferroptosis by eliminating ROS [44,45,91–94]. Then, we identified various compounds that provide neuroprotection in neonatal HIE models by ameliorating oxidative stress and ferroptosis by activating Nrf2-related pathways or the Nrf2 signaling pathway.

A recent study has shown that resveratrol, which is a nonflavonoid polyphenol compound, can exert a neuroprotective effect against ROS-induced ferroptosis in the brain tissue of neonatal rats after HI via the SIRT1/Nrf2/Gpx4 signaling pathway. Intraperitoneal administration of resveratrol upregulated the expression of Gpx4 and Nrf2 and reduced the iron levels and MDA concentration. Furthermore, resveratrol attenuated brain atrophy, memory impairment and cognitive impairments [44]. In addition, it has been demonstrated that exogenous melatonin (Mel), which is secreted by the pineal gland, upregulates the transcription of p-Akt, Nrf2 and Gpx4 and exerts neuroprotective effects by attenuating ROS-induced ferroptosis in the cerebral tissue of neonatal rats post-HI. Exogenous Mel (10 mg/kg) increased the GSH expression level and decreased the 4-hydroxynonenal (4-HNE) level. Additionally, treatment with exogenous Mel improved memory and long-term learning abilities in neonatal rats following HI [49].

Cai *et al.* [50] found that vitamin D (VD), which is a pleiotropic steroid hormone, could obviously suppress oxidative damage and ferroptosis via the Nrf2/HO-1 signaling pathway in both *in vivo* and *in vitro* HI models. VD significantly reduced the levels of MDA & ROS and the levels of inflammatory factors, including IL-1β, TNF-α and IL-6, and increased the activity of SOD & GSH, Nrf2, HO-1 and Gpx4. Furthermore, VD effectively alleviated mitochondrial damage and brain tissue injury caused by HI.

Overall, these compounds significantly inhibited ROS-induced ferroptosis via activation of the Nrf2 signaling pathway or Nrf2-related pathways and are promising neuroprotective drugs for neonatal HIE.

5. Conclusions

The reviewed literature in this study demonstrates that there is an important relationship between oxidative stress and the pathophysiology of neonatal HIE, and the Nrf2 signaling pathway plays an important part in cellular antioxidant defense. Moreover, compounds targeting oxidative stress via activation of the Nrf2 signaling pathway or Nrf2-related pathways exert great neuroprotective effects against neonatal HIE. However, there are crucial issues that should not be ignored. First, *Nrf2* mRNA was significantly upregulated post-injury in the cortex at 48 and 72 h and in the hippocampus at 48 and 72 h and 1 week in a mouse model of focal traumatic brain injury [95]. So, it will be better to confirm the optimal time to administer a possible Nrf2 activating compound via determining the spatial and temporal distribution of the Nrf2 signaling pathway in neonatal HIE. Furthermore, clinical and systematic preclinical studies are needed to support the safety of these compounds in the treatment of neonatal HIE. Second, a previous study has shown that due to the aggravation of oxidative stress, mitochondrial genome (mtDNA) expression is markedly higher in healthy controls than in patients with ischemic stroke [96]. Therefore, the relationship between neonatal HIE and ROS-induced mtDNA damage is worth exploring. Third, Liu *et al.* [97] reported that arachidonyl-2-chloroethylamide (ACEA) attenuated neurological dysfunction and oxidative stress by promoting mitochondrial autophagy (mitophagy) in an subarachnoid hemorrhage (SAH) model. Therefore, ROS-induced abnormal mitophagy may be an important factor in neonatal HIE. At last, ketogenic diet (KD) is one of treatments for neonatal HIE [98]. And a recent study has shown that KD upregulates the expression of β-hydroxybutyrate and ameliorates iron deposition by activating Nrf2-induced ferroptosis pathway on Alzheimer's disease model mice [99]. Similarly, Sechi *et al.* [100,101] have indicated that thiamine and its derivatives provide neuroprotection on neonatal HIE. And recent studies have suggested that thiamine may attenuate oxidative stress burden and inflammation by regulating the Nrf2-NF-κB signaling pathway [102,103]. Therefore, KD and thiamine should be further explored in neonatal HIE. In conclusion, the activation of Nrf2 may be a novel strategy for the prevention and treatment of neonatal HIE.

Abbreviations

Akt, protein kinase B; ACEA, arachidonyl-2-chloroethylamide; ARE, antioxidant response element; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; COX-2, cyclooxygenase-2; Cytc, Cytochrome c; CAT, catalase; CGA, chlorogenic acid; DLG, Asp-Leu-Gly; DAMPs, damage-associated molecular patterns; ETGE, Glu-Thr-Gly-Glu; EA, echinocystic acid; EGCG, epigallocatechin gallate; GPR39, G-protein-coupled receptor 39; Gpx, glutathione peroxidase; GSH, glutathione; Gpx4, glutathione peroxidase 4; h, hour; HIE, hypoxic-ischemic encephalopathy; HO-1, heme oxygenase-1; HIBD, hypoxic-ischemic brain damage; HIOC, N-[2-(5-hydroxy-1H-indol-3-yl) ethyl]-2-oxopiperidine-3-carboxamide; HI, hypoxic-ischemic; HIF-1α, hypoxia-inducible factor-1α; IL-1β, interleukin-1β; ICAM-1, intercellular adhesion molecule-1; IL-18, interleukin-18; IL-10, interleukin-10; IL-6, interleukin-6; IFN-γ, interferon-gamma; IL-1α, interleukin-1alpha; iNOS, inducible nitric oxide synthase; KD, ketogenic diet; Keap1, kelch-like ECH-associated protein 1; LDH, lactate dehydrogenase; LCA, licochalcone A; Lyc, lycopene; Mcl-1, myeloid cell leukemia-1; MDA, malondialdehyde; MnSOD, manganese-superoxide dismutase; MOR, morroniside; Mel, melatonin; mtDNA, mitochondrial genome; mitophagy, mitochondrial autophagy; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-kappa B; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; Neh1-7, Nrf2-ECH homology 1-7; NLRP3, the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; OGD/R, oxygen-glucose deprivation/reoxygenation; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PI3K, phosphatidylinositol 3-kinase; pro-IL-18, pro-interleukin-18; RXRα, retinoid X receptor-alpha; ROS, reactive oxygen species; SD, Sprague-Dawley; SFN, sulforaphane; SOD, superoxide dismutase; sMaf, small musculoaponeurotic fibrosarcoma; SIRT1, sirtuin 1; TNF-α, tumor necrosis factor α; TBHQ, tertiary-butylhydroquinone; TLRs, toll-like receptors; TGF-β, transforming growth factor-β; VD, vitamin D; β-TrCP, β-transducin repeat-containing protein; 4-HNE, 4-hydroxynonenal; 8OH-dG, 8-hydroxy-2′-deoxyguanosine.

Author Contributions

J-WM conceptualized and designed the study. J-XL and DZ wrote the manuscript. J-XL, DZ, LC, SC and J-WM designed the figures. J-WM revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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