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Prodeath or Prosurvival: Two Facets of Hypoxia Inducible Factor-1 in Perinatal Brain Injury

Wanqiu Chen, Robert P. Ostrowski, Andre Obenaus^{*}, and John H. Zhang

Departments of Physiology and Neurosurgery, Loma Linda University, Loma Linda, California, USA

^{*} *Department of Radiation Medicine, Loma Linda University, Loma Linda, California, USA*

Abstract

Hypoxia, which occurs in the brain when oxygen availability drops below the normal level, is a major cause of perinatal hypoxic-ischemic injury (HII). The transcriptional factor hypoxia inducible factor-1 (HIF-1) is a key regulator in the pathophysiological response to the stress of hypoxia. Genes regulated by HIF-1 are involved in energy metabolism, erythropoiesis, angiogenesis, vasodilatation, cell survival and apoptosis. Compared with the adult brain, the neonatal brain is different in physiological structure, function, cellular composition and signaling pathways related gene activation and response after hypoxia. The purpose of this review is to determine if developmental susceptibility of the brain after hypoxic/ischemic injury is related to HIF-1 α , which also plays a pivotal role in the normal brain development. HIF-1 α regulates both prosurvival and prodeath responses in the neonatal brain and various mechanisms underlie the apparent contradictory effects, including duration of ischemic injury and severity, cell-types, and/or dependent on the nature of the stimulus after HII. Studies report an excessive induction of HIF-1 in the immature brain, which suggests that a cell death promoting role of HIF may prevail. Inhibition of HIF-1 α and targeted activation of its prosurvival genes appear as a favorable therapeutic strategy. However, a better understanding of multifaceted HIF-1 function during brain development is required to explore potential targets for further therapeutic interventions in the neonate.

1. Introduction

Perinatal hypoxic-ischemic injury (HII) is the most important cause of acute mortality and morbidity in newborns (Volpe, 2001). Following HII, approximately 45% of newborns die or have permanent neurological deficits including cerebral palsy and there are currently no effective therapies. In contrast to adult stroke, the neonate exhibits a different response to injury. These age-dependent mechanisms of neuronal death result in differential long term neurological consequences and outcomes.

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor that is a pivotal regulator of oxygen homeostasis. Downstream many HIF-1 target genes play vital roles in developmental and physiological processes, such as angiogenesis, glucose transport, and cell proliferation/survival. Activation of these genes by HIF-1 can lead to either prosurvival or prodeath effects,

Corresponding author: John H. Zhang M.D., Ph.D., Department of Neurosurgery, Loma Linda University Medical Center, 11234 Anderson Street, Room 2562B, Loma Linda, California 92354, Telephone: 909-558-4723, Fax: 909-558-0119, Email address: E-mail: johnzhang3910@yahoo.com.

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and as such is important for the development of novel therapeutic interventions. However, before exploring the dichotomy of HIF-1 activation it is important to understand its role in development and perinatal brain injury.

2. Different vulnerability of developing brain compared with adult brain

Compared with the adult brain, the neonatal brain is different in physiological structure, function, cellular composition and signaling pathways related gene activation and protein expression. Likewise, the response to brain injury is also significantly different compared to the adult. First, the blood-brain barrier (BBB) has been shown to be more permeable to various blood-borne solutes and small lipid-insoluble molecules in the fetal rat brain than in adults (Stewart and Hayakawa, 1994; Dziegielewska et al., 1979). Second, the response to the injury and the primary mechanisms of neuronal cell death after the brain injury are different in the immature brain compared with those in the adult brain, which may exhibit a shift from a caspase-3-mediated to a noncaspase-3 mediated neuronal death. For example, the immature brain can accumulate more hydrogen peroxide (H_2O_2) than in adult brain (Lafemina et al., 2006), and activate mast cell migration after hypoxia-ischemia (Jin et al., 2007). Third, the neonatal brain responses to hypoxia or neuroprotective treatments are different compared to the mature brain. Simvastatin-dependent activation of eNOS (endothelial Nitric Oxide Synthase) has been cited as one of the main mechanisms responsible for neuroprotection in the adult brain, while eNOS activity is not affected by simvastatin treatment in the neonate (Cimino et al., 2005). Also, overexpression of superoxide dismutase 1, a known neuroprotectant in adult stroke models, is not protective after HII in the neonatal mouse brain (Sheldon et al., 2008).

The immature brain is more resistant to hypoxia/ischemia than the mature brain, which could be HIF-1-related. Lai et al. suggested that chronic postnatal hypoxia in different ages induces differential effects on brain glycolytic and TCA cycle enzymes activities, which is likely to be mediated by HIF-1 (Lai et al., 2003). Trollmann et al. reported the differential expression of HIF-1 and HIF-1-regulated target genes in the different age of developing brain upon exposure to hypoxia, which implies to be involved in the various cellular susceptibility to hypoxic distress (Trollmann et al., 2008). Resnik et al. reported that HIF-1 α activity differs with respect to both oxygen sensitivity and developmental stage. They showed that in fetal pulmonary artery (PA) smooth muscle cells (SMC), fetal HIF-1 protein levels were O₂-insensitive, however in adult PA SMC, hypoxia increased HIF-1 protein expression (Resnik et al., 2007).

Magnetic resonance imaging (MRI) has proved to be a valuable tool for monitoring development and pathology in the neonatal brain (Ashwal et al, 2007). Within hours to days after HII, MRI shows increased signal intensity in the cortex and striatum using diffusion- (DWI) and T2-weighted imaging (T2WI) (Figure 1). Compared to the adult, there is a basal level of HIF-1 activation present in the normal neonate, which is further increased after HII. A recent MRI study showed excellent correlation between neuroimaging and HIF-1 expression levels suggesting that MRI could be a potential surrogate marker (Jordan et al, 2005).

3. HIF-1: regulation of activity and target genes

HIF-1 is the most important factor regulating O₂ homeostasis and the cellular response to hypoxia and was purified first by Wang and Semenza in 1995 (Wang et al., 1995a). It is a heterodimeric transcription factor composed of an oxygen-regulated HIF-1 α and a constitutively expressed HIF-1 β subunit. During normoxic conditions, the hydroxylation of prolines in the oxygen-dependent degradation domain (ODD) of HIF-1 α will lead to degradation, while the degradation of HIF-1 α can be blocked if oxygen availability is reduced (Figure 2). Asparaginyl hydroxylation mediated by factor inhibiting HIF-1 (FIH) regulates HIF

transactivation, while prolyl hydroxylation by prolyl hydroxylase (PHD) regulates degradation of HIF-1 α chain. As illustrated in Figure 2, modification of HIF-1 by PHD and FIH, would lead to graded levels of expression but the co-activator p300 is required for full transcription of genes related to HIF-1. During hypoxic conditions, HIF-1 binds to the core DNA sequence 5'-G/ACGTG-3' within promoter/enhancer of its target genes and regulates their expression (Wang et al., 1995a).

Other than hypoxia, there are also a number of non-hypoxic stimuli which are capable of up-regulating this transcription factor, including growth factors, cytokines, free radicals and hormones. Major activators and inhibitors of HIF-1 activity are presented in the Table 1. Regulation of HIF-1 activity includes: oxygen sensing, redox status, and a variety of molecular factors and pharmacological agents (Table 1). Thus, HIF-1 is uniquely suited for bidirectional activation of transcription factors, with prodeath and prosurvival functions.

4. Role of HIF-1 in responses of developing brain to injury

The developing brain needs to effectively adapt to reduced tissue oxygen tension. mRNAs of HIF-1 α and HIF-1 β are constitutively expressed throughout the normal adult rat (Bergeron et al., 1999; Wiener et al., 1996) and in the embryonic mouse brain (Wang et al., 1995a). HIF-1 α is strongly expressed in the normal brain of fetus with a peak at E19 (Royer et al., 2000). However, HIF-1 α protein expression is found in the prenatal and perinatal brain but not in the normal adult brain (Figure 1). Based on differences between the neonatal and adult brain and HIF-1 function in neonatal and adult HII the response to therapeutic modulation of HIF-1 should also be different. First, HIF-1 α is essential for vascular development during embryogenesis and pathogenesis and also plays an important role in brain development. Complete deficiency of HIF-1 α leads to developmental arrest and lethality by E11, with neural tube and cardiovascular malformations and cell death within the cephalic mesenchyme (Iyer et al., 1998). Second, the HIF-1 to modulate HIF-1 in different age of animals. Third, the downstream events regulated by HIF-1 would also be expected to have differential outcomes in the neonatal and adult brain after HII. HIF-1 dependent activities including angiogenesis, inflammation, apoptosis, energy metabolism, and vasomotor tone etc, will be discussed below. Fourth, apart from being involved in normal brain development HIF-1 can affect many aspects of perinatal brain injury and often assumes dual cellular roles, those of prodeath and prosurvival. In adult mice with a late-stage deletion of HIF-1 α in the brain, the predominant role in acute hypoxia is proapoptotic (Helton et al., 2005). However, in neonatal stroke with moderate ischemia – reperfusion, HIF-1 α activation was neuroprotective (Mu et al., 2005). The mechanisms of HIF-1 α in mediating prodeath or prosurvival responses are not clear but is likely to play a significant role.

HIF-1 α expression in neonatal vs. other stroke models

The activation of HIF-1 by hypoxia occurs in a time and dose dependent manner both in vivo (Yu et al., 1998) and in cell culture (Jiang et al., 1996; Wang et al., 1995a). HIF-1 α is differentially regulated depending upon the different types of stimuli, severity of the insult and the age of the animal.

In the neonatal brain, hypoxia only, is sufficient to regulate multiple molecular mediators that may contribute, but are not sufficient, to induce long-term neuronal damage (van den Tweel et al., 2006). When hypoxia is accompanied by a concomitant reduction in blood flow (ischemia) are there more persistent alterations of HIF-1 expression. In 10-day-old pups after 1.5hr middle cerebral artery (MCA) occlusion, HIF-1 α protein peaks at 8 hr and declines subsequently at 24 hr in the injured cortex (Mu et al., 2003). In a postnatal 12-day rat HII model, van den Tweel et al. reported that HIF-1 α proteins increased and peaked at 3 hr but returned to baseline at 6 h after injury (van den Tweel et al., 2006). In newborn rats the cerebral

cortex and hippocampus are major sites of HIF-1 α mRNA expression (Bergeron et al., 1999). A study by Kaur et al. showed that an enhanced mRNA and protein expression of HIF-1 α together with its target genes such as VEGF, eNOS, iNOS and nNOS in the neonatal hippocampus as early as 3 h following hypoxia, which may be responsible for causing damage to the dendrites and axons in the immature hippocampus (Kaur et al., 2008). The signaling pathways responsible for the regulation of HIF-1 α in the developing brain after HI have been studied. Recent reports suggest that Erk 1/2 MAPK and PI3K/Akt pathways are involved in HIF-1 α activation after neonatal HII (Li et al., 2008a; Li et al., 2008b). NF- κ B is also shown to mediate HIF-1 α transcription (Rius et al., 2008).

In adult rats, focal cerebral ischemia induced a robust upregulation of mRNAs encoding HIF-1 α in the peri-infarct penumbra by 7.5 hr after ischemia with a 15–17-fold increase of HIF-1 message that continued for 20 hr. Another study reported a 1.8–2.5 fold increase in both HIF-1 α and HIF-1 β proteins by 20 hr after reperfusion (Bergeron et al., 1999; Sharp et al., 2001). However, the durability of HIF-1 induction has been noted in global ischemia. In the 2-VO global ischemic model, HIF-1 α expression was increased 8–24 hr after ischemia and persisted for 96 hr after reperfusion (Jin et al., 2000; Li et al., 2005). The prolonged HIF-1 α accumulation may be induced by additional factors during the delayed reperfusion period, such as insulin-like growth factor-1 (IGF-1), as it takes only several hours to revive tissue from hypoxia after reperfusion (Chavez and LaManna, 2002). Meanwhile, thrombin has been implicated as a non-hypoxic regulator of HIF-1 α in cultured smooth-muscle cells (Gorlach et al., 2001). Perihematomal HIF-1 α protein has been shown upregulated at 3 days after intracerebral hemorrhage without changing mRNA levels, and hirudin reduced HIF-1 α upregulation in response to the presence of both thrombin and blood (Jiang et al., 2002). The expression of HIF-1 α was shown to closely correlate with the duration of hemorrhage and the amount of bleeding and its subsequent ability to induce apoptosis of neurons (Zhu et al., 2004). Additionally, pretreatment with a low dose of thrombin can reduce brain edema after hemorrhagic stroke, which was also thought to be associated with increasing HIF-1 α levels and alterations in two HIF-1 α target genes, transferrin (Tf) and transferrin receptor (TfR) in the brain (Hua et al., 2003).

Apoptosis in perinatal brain injury

HIF governs a number of apoptotic genes including the Bcl-2/adenovirus E1B 19kDa-interacting protein 3 (BNIP3), NIX (also called BNIP3L) and NOXA. Of these, BNIP3 has been demonstrated to contribute to cell injury in the developing cortex (Sandau and Handa, 2007) via a caspase-independent cell death mechanism. Although it is known that in neonatal brain the process of apoptosis is largely caspase-3 dependent, Yin and others showed that targeting both types of apoptosis is a reasonable therapeutic option for neonatal brain injury (Yin et al., 2006). Moreover, promoter analysis of rat caspase-9 and caspase-3 showed HIF-1 binding elements (Nishiyama et al., 2001; Liu et al., 2002).

p53 is also involved in HIF-1 governed apoptosis after induction of HII. p53 induction by HII binds with HIF-1 α and results in stabilization within cerebral tissues. Neonatal HII results in HIF-1 α dephosphorylation with a similar pathological phenotype underlying cell death via the p53 pathway (Calvert et al., 2006). Additionally, p53 largely contributes to an inflammatory response in the neonatal brain (Eklind et al., 2006). Suppressing HIF-1 α with hyperbaric oxygen treatment remarkably attenuated HIF-1 α and p53 interactions with reduced apoptosis after neonatal hypoxia-ischemia (Calvert et al., 2003; Calvert et al., 2006).

BBB, edema and angiogenesis

Cerebral microvascular endothelial cells form the BBB and its tight junctions are critical for maintaining brain homeostasis and low permeability. Ischemia/reperfusion is known to damage

these tight junctions and lead to increased permeability changes. Activation of HIF-1 α in the adult brain of dystrophic mice coupled with VEGF and VEGFR-2 up-regulation in neurons and in endothelial cells and is correlated with changes to endothelial ZO-1 and claudin-1 expression, which might contribute to both BBB opening and increased angiogenesis (Nico et al., 2007). HIF-1 α responses upon hypoxia in the developing brain are critically involved in vasculogenesis, neural differentiation, and brain plasticity (Curristin et al., 2002). Reports also showed that HIF-1 α mediates neural progenitor cell proliferation and differentiation (Zhao et al., 2008; Kim et al., 2008). When rats at different ages are exposed to HII, younger animals (P7) had a leakier BBB, compared with older animals (P21). Therefore only in immature animals does the severity of injury correlate with BBB opening (Muramatsu et al., 1997). No benefit of reducing BBB permeability was observed even in P10 rats, which additionally may suggest that alleviation of brain edema is not sufficient for improvement of outcomes in face of an excess hypoxic signaling (Wang et al., 2007). Inhibition of HIF-1 α accumulation in the neonatal brain by 2-methoxyestradiol protected the BBB from HII which might be related to a reduction of VEGF expression in the early stage (Chen et al., 2008). Additionally, VEGF through proinflammatory actions, such as enhanced adhesion of leukocytes to vascular walls, may be involved in periventricular white matter damage as demonstrated in 1-day old rats (Kaur et al., 2006).

The expression of water channels in HII has received some attention possibly due to the very acute induction of cytotoxic edema in neonates (Badaut et al., 2007). The level of edema was found not to correlate with the greatest level of aquaporin (AQP) 4 expressions in the neonate brain (Badaut et al., 2007). Regions bordering the ischemic cortex and striatum might be considered for therapeutic modulation of AQP expression and could reduce edema expansion in HII. An in vitro study showed that AQP4 promotes water flux into astrocytes during HI, whereas it promotes water flux out of astrocytes with reoxygenation, which may serve as a target during different periods of hypoxic-ischemic brain edema in neonates (Fu et al., 2007). The role of HIF-1 in aquaporin regulation has not yet to be examined, but in adult mice with predominant brain deletion of HIF-1 α the gene for AQP4 was upregulated (Helton et al., 2005). It has been shown that the promoter of AQP5 also possesses HRE and is regulated by HIF-1 α (Zhou et al., 2007).

Recent studies have demonstrated that activation of HIF-1 leads to upregulation of factors that are essential for blood vessel formation and it is one of the primary forces driving both physiological and pathological angiogenesis (Ziello et al., 2007). Angiogenesis-related genes, such as VEGF, VEGFR-1, plasminogen-activator inhibitor-1 (PAI-1), are all target genes of HIF-1 α . Increased expression of mRNA of HIF-1 α and VEGF precedes angiogenesis in brain of P7 rats (Huang et al., 2004). Anan and colleagues showed angiogenesis can be induced by injecting naked DNA for HIF-1 α , HIF-2 α or COX-2 into the brain, which suggested that neovascularization may be indirectly achieved using a transcriptional and cytokine regulatory strategies (Anan et al., 2006). Therefore, the acute effects of VEGF, leading to increased BBB permeability, should be distinguished from its latent effects, that include neovascularization and prosurvival actions.

Inflammation (COX-2, iNOS)

Inducible NOS (iNOS) and cyclooxygenase 2 (COX-2) are HIF-1 target genes which contribute to brain injury via inflammation induction (Kunz et al., 2007; Mi et al., 2007). It has been demonstrated that decreased expression of COX-2 is associated with reduced injury in the neonatal brain (Yin et al., 2007) whereas enhanced COX-2 expression (and iNOS) accompanies neuronal loss after HII (Yang et al., 2005). Recent studies with COX-2 inhibitors showed a reduction of brain injury in neonates and a subsequent amelioration of neurobehavioral outcomes. This effect appeared durable, suggesting an effective protection of the neurovascular

unit (Fathali et al., 2007). Upon hypoxia, HIF-1 is involved in upregulation of COX-2 in endothelial cells, which catalyzes production of eicosanoids (Cook-Johnson et al., 2006). A strong iNOS upregulation mediated by HIF-1 was detected after focal cerebral ischemia in the adult brain (Suzuki et al., 2002; Matrone et al., 2004). However, somewhat conflicting data exists on the involvement of iNOS in neuroinflammation after neonatal HII. Major cellular sources of iNOS are astrocytes and microglia, both activated after neonatal HII (Bal-Price and Brown, 2001; McRae et al., 1996). Recently one group reported only marginal expression of iNOS in P12 rats after HII (Suzuki et al., 2002; van den Tweel et al., 2005). Meanwhile, expression of iNOS was found to be unchanged after HII (Blumberg et al., 1999) or even significant decrease of iNOS mRNA after hypoxia in both P0 and P7 age mice (Trollmann et al., 2008). In contrast, other authors have clearly demonstrated that excess upregulation of iNOS may contribute to the neonatal brain injury and a selective iNOS antagonist proved effective in neonatal stroke (Ikeno et al., 2000; Tsuji et al., 2000). It should be however emphasized that whereas 3-nitrotyrosine may contribute to the mechanism of brain damage after HII, an excess NO produced upon iNOS activation can kill neurons by acute inhibition of mitochondrial respiration at cytochrome oxidase (Mander and Brown, 2004).

Vasomotor tone (endothelin-1, HO-1)

Endothelin-1 (ET-1) is a HIF-1 target gene (Wykoff et al., 2000). Its injurious potential has been demonstrated in a neonatal model of epilepsy (Mateffyova et al., 2006). ET-1 was found elevated in the amniotic fluid and suggested to be a predictive marker for perinatal hypoxia (Xu et al., 1999). Widespread astrocytic and cerebral endothelial induction of ET-1 mRNA was observed in a perinatal hypoxic-ischemic model (Tsang et al., 2001). Although ET-1 seems to contribute to perinatal cerebral hypoperfusion the actual impact of hypoxia and HIF-1 signaling is complex. The vascular contractility to ET-1 is countered by HO-1 activation in hypoxia (Govindaraju et al., 2005; Jernigan et al., 2001). HO-1 is also a HIF-1 target gene, with a multiple regulatory binding sites in its promoter (Jones and Bergeron, 2001). It is upregulated after newborn HII but not by hypoxia alone (Bergeron et al., 1997), thus its expression correlates with the severity of brain insult. HO-1 elevation in macrophages may contribute to tissue vulnerability after HII in neonates possibly through a release of several cytotoxic molecules and local production of bilirubin (Bergeron et al., 1997).

5. The multifaceted role of HIF-1 in brain protection

Prosurvival or prodeath?

HIF-1 is considered as a regulator of both prosurvival and prodeath responses in the central nervous system (Table 2, Figure 3). As Figure 3 suggests, both hypoxia and free radicals can activate HIF-1 and the opposing effects of HIF-1 α can be attributed to the duration and severity of hypoxia and free radical levels. Mild hypoxia induces adaptive gene expression such as EPO, Glut1 and VEGF, whereas severe or sustained hypoxia HIF-1 α can lead to activation of prodeath genes, such as BNIP3, COX2, or p53 stabilization. In addition, with decreasing levels of pO₂ during ischemia there are subsequent increases in reactive oxygen species (ROS) that may also trigger secondary transcription factors responsible for ensuing cell death. In neonatal HII, cumulative HIF-1 activation, due to prolonged hypoxia and excess of ROS (immature antioxidant defenses) combined with basal hypoxic signaling, may trigger prodeath responses overpowering the effects of prosurvival genes. Several studies have shown support for HIF-1 mediating a prosurvival response. Firstly, stimulation of HIF-1 upon preconditioning by hypoxia or induction of HIF-1 was important for the induction of prosurvival genes (EPO, VEGF etc) resulting in the survival of cells (Bergeron et al., 2000; Digicaylioglu and Lipton, 2001; Sharp et al., 2004; Stenzel-Poore et al., 2003). Preconditioning with CoCl₂ or deferoxamine (DFX) 24 hr before hypoxia-ischemia resulted in 75 and 56% brain protection, respectively (Bergeron et al., 2000). DFX pretreatment ameliorated hippocampal neuronal

death after in vitro oxygen-glucose deprivation (OGD) and the protection could be reversed with transfection of anti-HIF-1 (Ferriero, 2005). These beneficial effects could be mediated by an increase of HIF-1, although the antioxidant effects of DFX are also likely to contribute (Freret et al., 2006). Secondly, HIF-1 target genes such as EPO and VEGF have been shown to exhibit neuroprotective effects (Ferriero, 2005). Thirdly, HIF-1-induced angiogenesis and glycolytic metabolism results in increased delivery of oxygen and nutrition that is critical for cell survival under hypoxic/ischemic conditions (Bergeron et al., 2000).

In contrast, HIF-1 also has been shown to mediate prodeath responses. First, HIF-1 has been shown to interact with p53 and promote neuronal cell death as p53-dependent apoptosis (Halterman et al., 1999; Renton, 2003). Meanwhile, embryonic stem cells deficient in HIF-1 failed to induce p53 and increase resistance to stress-induced cell death (Carmeliet et al., 1998). HIF-1 has been also shown in vitro to mediate hypoxia-induced growth arrest and apoptosis (Goda et al., 2003). A negative form of HIF-1 protected cortical neurons from delayed death following HII in vitro (Halterman et al., 1999). Secondly, HIF-1 can regulate the expression of BH3-only pro-apoptotic family members such as BNIP3. Several studies have shown that BNIP3 is increased following cerebral ischemia (Althaus et al., 2006; Guo et al., 2001; Schmidt-Kastner et al., 2004). Third, in vivo HIF-1 knockout studies showed that conditional or late-stage brain depletion of HIF-1 could be neuroprotective following acute global ischemic injury (Helton et al., 2005) and genes involved in apoptotic pathways were down-regulated in these HIF-1 knockout mice (Helton et al., 2005). Another study, however, found that neuron specific knockdown of HIF-1 α resulted in increased tissue damage after focal cerebral ischemia (Baranova et al., 2007). Fourth, cloning of the rat caspase-3 gene promoter demonstrated the existence of a functional HIF-1-binding element in this promoter (Liu et al., 2002). Also, ischemia increased HIF-1 binding-activity on the caspase-3 gene (Van Hoecke et al., 2007). After focal ischemia, colocalization of procaspase-3 cleavage and HIF-1 α expression was found in the penumbra and even unlesioned tissue. It suggested a causative relationship between HIF-1 α and caspase-3 induction through functional binding activity to the caspase-3 gene promoter (Van Hoecke et al., 2007). Others have suggested that the caspase-3/HIF-1 response may be dependent on the duration and severity of the ischemic injury (Halterman and Federoff, 1999). Mild hypoxia induced adaptive gene expression can occur transiently or persistently depending on the duration of the stimulus, whereas severe or sustained hypoxic conditions HIF-1 can promote apoptotic cell death. The differential response of HIF-1 to injury could be stimulus dependent. HIF-1 responded differently to oxidative death induced by glutathione depletion vs. DNA damage by camptothecin in a hippocampal neuroblast cell line (Aminova et al., 2005).

The role of HIF-1 α in mediating both prodeath and prosurvival responses, is dependent on the duration (Halterman and Federoff 1999) and types of pathological stimuli (Aminova et al. 2005) as well as the cell type that it is induced in (Vangeison et al. 2008) (Table 2). Recently Baranova *et al.* reported there are two phases of HIF-1 α activation after cerebral ischemia (Baranova et al., 2007). The first phase occurred immediately after injury till 12 hr, which correlated with the upregulation of most pro-death genes, while in the second phase of HIF-1 α activation, most pro-survival genes are involved (Baranova et al., 2007). It suggests that HIF-1 α may play different role during the acute and chronic phases after ischemia.

Targeting HIF-1 and its genes for novel therapies of neonatal stroke

Inhibition of HIF at selected targets is a promising therapeutic approach for neonatal HII. Studies with genetically engineered animals and siRNA therapeutics are definitely warranted and should include careful long-term evaluation, given the significance of HIF in brain development. In clinical settings, however, there is a need for pharmacological tools that could be easily administered and would penetrate BBB. Many substances that show promise in cell

culture cannot effectively penetrate and often require specially designed vectoring systems. Nano particles-based systems are an emerging choice (Kingsley et al., 2006). Also, chemical modification of the compound itself may lead to improved brain protection. For example, when compared with EPO, carbamylated EPO produced more general and more efficient neuroprotective effect (Montero et al., 2007). Promising results were found for the pharmacological HIF-1 inhibitor 2-methoxyestradiol (2ME2) in neonatal HII, when administered immediately after the brain insult 2ME2 reduced injury in the immature brain (Chen et al., 2008).

6. Final remarks

The complex response of the neonatal brain to injury may lie in a profuse and excessive induction of HIF-1 and other molecular factors throughout the brain. There are reports of HIF-1 α and other molecular factors equally being induced in infarcted and non infarcted hemispheres in the neonatal HII (van den Tweel et al., 2006). Although a plausible interpretation is that molecular mediators alone are not sufficient to induce long-term neuronal damage further studies are needed to examine a mechanism by which sustained HIF-1 activation is possible and whereby discrete damage occurs to synaptic apparatus or cell organelles in non-infarcted tissues.

Besides, most frequently used models of neonatal hypoxia ischemia may not be perfectly accurate for modeling clinically relevant perinatal brain injury. Duration of hypoxia affecting human immature brain in many cases is longer than 90–120 min commonly used in animal models. Clinically, HII in some cases occurs in utero and extends into the postnatal period (Perlman, 2006).

It has been postulated that the duration of hypoxia correlates with the period of time during which HIF remains elevated in the cells (so called “memory of HIF”) (Kamat et al., 2007). In a prolonged maternal hypoxia model, cellular injury is barely detectable in neonate brains at birth, however significant disturbances of brain architecture, and impaired brain function are seen months after the insult (Golan et al., 2004a; Golan et al., 2004b). Therefore, given that HIF-1 has the potential to carry prodeath signals, its role in chronic and latent responses to perinatal brain injury need to be further explored.

7. Conclusion

The developmental susceptibility of the brain after hypoxic/ischemic injury is likely to be related to HIF-1 α , which plays a pivotal role in the development of injury and in normal brain development. To explore potential targets for further therapeutic intervention a better understanding of HIF-1 α function, the importance of its target genes during the developmental stage, and further identification of fundamental differences between the neonate and adult brain is required.

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Figure 1. Comparison of HIF-1a staining in the neonate and adult rat

Magnetic resonance imaging of the neonate after HII demonstrates increased signal intensity in the cortex and striatum using diffusion-(DWI) and T2-weighted imaging (T2WI). In the cortex of control neonates there is a significant basal level of HIF-1a that is localized to neurons (arrow). After neonatal HII and other insults there is an increased level of expression within the cortex of the neonatal brain that is clearly localized to neurons and other cell types (arrows). However, in the control adult cortex there is no HIF-1 staining that is confirmed at higher magnification (arrows).

Figure 2. Molecular cascade responsible for HIF-1 activation

Under normal levels of pO₂ HIF-1 α is rapidly degraded via an ubiquitination pathway. Although not well studied, moderate levels of hypoxia are known to activate HIF-1 by a p300 independent pathway that results in limited gene transcription. However, hypoxia induces HIF-1 by activation of p300 and binding with both HIF-1 α and HIF-1 β that in turn activates a hypoxic response element (HRE). This molecular cascade results in full transcription of over 70 different genes of which a few general physiological processes are illustrated. Both activation and inhibition of HIF-1 occur via the actions of prolyl hydroxylase (PHD) and factor inhibiting HIF-1 (FIH). PHD is sensitive to O₂ levels for activation. PHD and FIH act to activate HIF but the co-activator p300 is required for full transcription of genes related to HIF-1.

Figure 3. The dual role of HIF-1 function in prodeath and prosurvival pathways is highlighted in this schematic

Both hypoxia and free radicals can activate HIF-1. As pO_2 decreases HIF-1 regulates transcription pathways that leads to activation of prodeath genes, for example, BNIP3, COX2 and others, or stabilization of p53. In addition, with decreasing levels of pO_2 during ischemia there are subsequent increases in reactive oxygen species (ROS) that may also trigger secondary transcription factors responsible for ensuing cell death. However, HIF-1 also plays a role in pro-survival pathways by transcriptionally activating protective genes such as EPO, Glut1 and VEGF. In neonatal HII, cumulative HIF-1 activation, due to prolonged hypoxia and excess of ROS (immature antioxidant defenses) combined with basal hypoxic signaling, may trigger prodeath responses overpowering the effects of prosurvival genes.

Table 1**Regulation of HIF-1 activity**

HIF-1 activator	Related Mechanisms	Ref.
Calcineurin	Promotes HIF-1 α expression by dephosphorylating RACK1 and blocking RACK1 dimerization	Liu et al., 2007
Cytokines – eg. IL-1 β , TNF-1 α	Induce HIF-1 α mRNA expression via PI3K pathway	Westra et al., 2007
Growth factors, eg. IGF-1	Prolonged HIF-1 accumulation	Chavez and LaManna, 2002
Hypoxia	Inhibition of HIF-1 α subunit rapid degradation	Huang et al., 1996
Iron chelators, eg. desferrioxamine	Inhibit hydroxylation of Proline in ODD domain	Ivan et al., 2001
ROS	Modulating upstream signaling pathways such as hydroxylases or phosphatases, or via NF-kappaB.	Bonello et al., 2007
Thrombin	Redox-sensitive cascade activated by ROS may be involved in this response.	Gorlach et al., 2001
Vitamin E	Induced the expression of the α subunit of HIF-1	Zhang et al., 2004
Sodium orthovanadate, tyrosine kinase	An inhibitor of tyrosine phosphatases increased the basal level of HIF-1 proteins and HIF-1 activity	Wang et al., 1995b
Mitogen-activated protein kinase (MARK)	MAPK may mediate HIF-1 activation by various molecules, including nitric oxide	Kasuno et al., 2004; Richard et al., 1999; Li et al., 2008
HIF-1 inhibitor	Related Mechanisms	Ref.
Guanylate-cyclase activators, eg. YC-1	YC-1 completely blocks HIF-1 α expression at the post-transcriptional level and linked with the oxygen-sensing pathway	Yeo et al., 2003
HIF prolyl 4-hydroxylases	Belongs to a family of iron- and 2-oxoglutarate-dependent dioxygenases that negatively regulate the stability of HIF-1	Siddiq et al., 2005
HSP90 inhibitor – 17-AAG	Inhibition and eventual degradation of Hsp90 thus destabilization of HIF-1	Neckers and Neckers, 2005
2-methoxyestradiol (2ME2)	Decreased nuclear HIF-1 α -binding activity and affected the expression of downstream genes	Ricker et al., 2004
Thioredoxin inhibitors	Decrease HIF-1 α functional transcriptional activity and DMA binding	Jones et al., 2006
Topoisomerase (Topo)-I inhibitors	Repression of HIF-1-dependent induction of gene expression	Rapisarda et al., 2002
Tyrosine kinases inhibitor, eg. genistein	Blocked the synthesis of both HIF-1 subunits as well as HIF-1 DMA-binding activity	Wang et al., 1995a

Table 2**Prosurvival or prodeath role of HIF-1**

Prosurvival role of HIF-1 and its related genes/proteins upon prosurvival response	Ref.
Preconditioning by hypoxia or reagent upregulates HIF-1alpha and protects against ischemic neonatal brain injury EPO, IGF-1, VEGF, Glut-1	Bergeron et al., 2000; Sharp et al., 2004; Hamrick et al., 2005
Some HIF-1-target genes may underlie neuroprotection EPO, VEGF	Digicaylioglu and Lipton, 2001; Ferriero, 2005
Cobalt reduces hypoxia oxidative stress by maintaining higher cellular HO-1 via HIF-1alpha Heme oxygenase 1 (HO-1)	Shrivastava K et al., 2008
Neuron-specific inactivation of HIF-1alpha increases brain injury post transient focal cerebral ischemia EPO, Glut-1, ENOLASE, VEGF, Flt-1, PAI-1, and Ang-2	Baranova et al., 2007
Prodeath role of HIF-1 and its related genes/proteins upon prodeath response	Ref.
HIF-1alpha signaling in primary cortical neurons elicits delayed cell death involving the stabilization and increase of p53 p53	Halterman et al., 1999
HIF-1alpha mediate hypoxia-induced growth arrest and apoptosis p21, p27, p53	Carmeliet et al., 1998; Goda et al., 2003
HIF-1alpha regulate the expression of pro-apoptotic proteins BNIP3, NIX	Althaus et al., 2006; Bruick, 2000; Sowter et al., 2001
HIF-1alpha mediates the induction of caspase-3 in the ischemic brain Caspase-3	Van Hoecke et al., 2007
Brain-specific knock-out of HIF-1alpha reduces hypoxic-ischemic damage	Helton et al., 2005
The role of HIF-1 is both prosurvival and prodeath	Ref.
Depends on hypoxic duration and severity	Halterman and Federoff 1999
Depends on different types of pathological stimuli	Aminova et al. 2005
Depends on different cell types	Vangeison et al. 2008