# Targeting Hypoxia Inducible Factor-1α: A Novel Mechanism of Ginsenoside Rg1 for Brain Repair after Hypoxia/Ischemia Brain Damage

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**Abstract:** Hypoxia/ischemia brain damage (HIBD) is one of the most common central nervous system insults in newborns. Brain repair following HIBD is closely associated with cellular processes such as cell survival, angiogenesis, and neurogenesis. In recent years, many studies have suggested that ginsenoside Rg1, one of the major active ingredients of ginseng, may increase neural viability, promote angiogenesis, and induce neurogenesis. However, there are few reports on roles of Rg1 in HIBD repair, and the mechanisms involved are unclear. Recently, a Chinese drug consisting of Rg1 has been shown to be a potential regulator of hypoxia-inducible factor-1α expression in HIBD. Since it has been shown that HIF-1α is a key transcription factor involved in the neuroprotective response to HIBD, it is possible that Rg1 could facilitate the process of brain repair, possibly modulating cell survival, angiogenesis, and neurogenesis after HIBD by targeting HIF-1α.

**Keywords:** Angiogenesis, cell survival, cellular signal pathway, ginsenoside Rg1, hypoxia inducible factor-1α, hypoxia/ischemia brain damage, neurogenesis.

## INTRODUCTION

Hypoxia/ischemia brain damage (HIBD) is one of the most common central nervous system (CNS) disorders occurring in newborns, with serious consequences such as cerebral palsy, learning disabilities, and behavioral disorders [1]. Therefore, research on the pathogenesis and treatment of HIBD is of great importance. A series of investigations has indicated that brain repair following HIBD is associated with enhanced cell survival, angiogenesis, and neurogenesis in and around the injured regions.

We have previously demonstrated that hypoxia-inducible factor-1α (HIF-1α) is involved in modulating many pathologic processes related to hypoxia/ischemia, including HIBD [2-6]. HIF-1α is the regulatory subunit of HIF-1, which controls transcription of various genes involved in processes such as energy metabolism, vasculogenesis, apoptosis and proliferation, cell migration and differentiation [2-4, 7]. Therefore, it is important to understand the mechanisms that potentially regulate HIF-1α, which may reveal novel strategies for HIBD treatment.

Ginseng, the root and rhizome of *Panax ginseng* C A Meyer (a Chinese herb), has been used as a tonic remedy in Chinese traditional medicine for over 2000 years. In the last decades, pharmacological effects of Ginseng on the CNS and cardiovascular system have been demonstrated [8]. With the development of modern technology, increasing numbers of active ingredients have been isolated and purified from this herb, and ginsenoside Rg1 (Rg1) has been identified as one of the major active ingredients. In recent years, many beneficial effects of Rg1 on the nervous and cardiovascular systems have been reported, included increasing neuronal cell viability [9], promotion of angiogenesis [10], and induction of proliferation and differentiation of neural progenitor cells or neural stem cells (NSCs) [11, 12].

In this review, we examine the evidence for biological effects of both HIF-1α and Rg1 on crucial processes related to brain repair following HIBD, and the potential relationship between Rg1 and HIF-1α. Furthermore, based upon this evidence we hypothesize a novel mechanism for this repair, which can provide new ideas to investigate to overcome neonatal HIBD.

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## HIF-1α AND HIBD

In our previous studies, we demonstrated that HIF-1α is involved in modulating many pathologic processes related to hypoxia/ischemia insults, including HIBD [2-6]. HIF-1α is the regulatory subunit of HIF-1, which is a nuclear factor that controls transcription of various genes involved in processes such as energy metabolism, vasculogenesis, apoptosis, proliferation, cell migration and differentiation [2-4, 7].

HIF-1α promotes the transcription of vascular endothelial cell growth factor (VEGF), which is associated with neo-vascularization following tissue hypoxia/ischemia. VEGF can activate a series of signal transduction pathways by interacting with its receptors on endothelial cells. Additionally, VEGF contributes to brain protection by enhancing survival, proliferation and differentiation of neural precursor cells [13] and by reducing cell apoptosis of vascular endothelial cells, neurons, and glia after hypoxia/ischemia [14].

HIF-1α is also involved in cellular apoptosis, which is closely related to mitochondrial function. The increase of mitochondrial membrane permeability during HIBD leads to the release of cytochrome c, which activates caspase-3 and finally induces apoptosis. Caspase-3 is a key regulator of cellular apoptosis. The active form of caspase-3 is cleaved caspase-3 (CC3), which causes specific DNA fragmentation, one of the characteristics of early neuronal cell apoptosis after brain hypoxia or ischemia. CC3 is a reliable and sensitive indicator of apoptosis. Our previous investigation demonstrated a negative correlation between HIF-1α and CC3 expression in a HIBD model of neonatal rats [2].

HIF-1α is involved in neurogenesis during HIBD repair, and its activity is essential for induction of erythropoietin (EPO) mRNA and protein expression under hypoxic conditions. Investigation by Shingo *et al.* [15] identified EPO as an autocrine–paracrine factor, capable of regulating the production of neural progenitor cells by forebrain NSCs. Later, our study demonstrated that EPO increased the percentage of newly generated neurons while decreasing newly generated astrocytes following neonatal stroke. These results

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suggest a beneficial effect for EPO in neurogenesis, which is essential for CNS repair following HIBD [16]. Milosevic *et al*. [13] found that conditional knock-out of HIF-1α in midbrain neural precursor cells leads to midbrain-specific impairment of survival and proliferation, and significant reduction of dopaminergic differentiation. Moreover, results from an *in vitro* study provided evidence that HIF-1α was involved in the regulation of dopaminergic differentiation of NSCs in lowered oxygen environments [7]. In 2008, Wu *et al*. [17] reported that NSCs differentiation could be promoted by HIF-1α after focal cerebral ischemia, thus improving functional recovery. It should be noted that expression of EPO was upregulated in this process. However, whether EPO is activated by drugs like Rg1 and thus mediates neurogenesis after HIBD is unknown. In consideration of the key role that HIF-1α plays in the repair of HIBD, it is very important to understand the mechanisms of regulation of HIF-1α, which may reveal novel strategies for HIBD treatment.

## PHARMACOLOGICAL EFFECTS OF RG1 ASSOCIATED WITH HIBD REPAIR

### Anti-Apoptosis Effects

The anti-apoptotic effect of Rg1 was first reported in 1997, when Rg1 was shown to concentration-dependently inhibit apoptosis of cultured cortical neurons induced by serum withdrawal [18]. Subsequently, *in vivo* and *in vitro* experiments revealed that neuronal cell apoptosis induced by various factors, such as hypoxia/ischemia stress, dopamine and its derivative 6-hydroxydopamine, and rotenone, could be reversed by Rg1 administration [9, 19-22]. Furthermore, these studies suggested that mechanisms underlying the anti-apoptotic effect of Rg1 involved decreasing nitric oxide and peroxynitrite contents, reducing intracellular calcium concentration, inhibiting the mitochondrial apoptotic pathway, activation of neurotrophic factors as well as anti-apoptosis Bcl family members, and inactivation of pro-apoptotic proteins such as Bad and caspase-3. However, the signal transduction pathways through which Rg1 modifies expression or activity of such factors are not clearly understood.

### Angiogenesis Effects

Regeneration and functional recovery of new blood vessels are vital for cellular repair after neonatal HIBD. Early studies suggested that Rg1 exerts angiogenic effects both *in vitro* and *in vivo* [23, 24]. Subsequently, Leung *et al.* [25] reported that Rg1 was a potent stimulator of VEGF in human endothelial cells. Recently, study of cultured endothelial progenitor cells showed Rg1 promoted their adhesion, proliferation, migration and *in vitro* vasculogenesis in a dose- and time-dependent manner. It should be noted that VEGF production was increased by Rg1 in this process [26]. These findings provide evidence that the Rg1-associated angiogenesis may be VEGF-dependent.

### Neurogenesis Effects

Activation of NSCs proliferation and directional differentiation are major events on which neurogenesis following HIBD depends. In 2003, an *in vivo* study showed that neurogenesis in the dentate gyrus was significantly enhanced following Rg1 treatment after global ischemia [19]. This effect may be attributed to not only increased cell proliferation, but also increased survival of newly generated neurons. Subsequent investigation by the same authors revealed Rg1 enhanced proliferation of rodent hippocampal progenitor cells both *in vitro* and *in vivo* [11]. Recently, *in vitro* experiments performed in China demonstrated that Rg1 could promote both proliferation of NSCs [27] and their ability to differentiate into neurons [12]. However, *in vivo* effects of Rg1 on biological characteristics of NSCs after hypoxia/ischemia and the possible mechanisms are not clear. Considering the results from the study of Wu *et al*. [17], it will be interesting to explore whether the effect of Rg1 on NSCs is associated with HIF-1α upregulation and its downstream modulation of factors such as EPO.

## MECHANISMS UNDERLYING NEUROPROTECTIVE EFFECTS OF RG1

As mentioned above, Rg1 may play a protective role in neonatal HIBD by affecting the expression of VEGF, CC3, and EPO, which are known to be regulated by HIF-1α. Therefore, it is not unreasonable to presume that the protective effects of Rg1 would be achieved, at least partly, by regulating HIF-1α. Recently, an *in vivo* study demonstrated that *Shenmai injection*, a medicinal compound consisting of *red ginseng*, could increase HIF-1α expression [28]. This study suggested a potential regulatory effect of Rg1 on the expression of HIF-1α because *red ginseng* also contains Rg1 [28]. Futhermore, if Rg1 does contribute to regulation of HIF-1α, how it transduces its effects is an issue that needs further study.

Our previous studies have demonstrated that both the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathway and extracellular signal-related protein kinase (ERK) pathways are involved in the regulation of HIF-1α in the developing rat brain after HIBD [5, 6]. Once HIF-1α is upregulated by PI3K/Akt and ERK activation in this setting, it can promote VEGF [5, 6], inhibit cellular apoptosis [2], and induce neurogenesis [7]. In addition to our findings, other reports provide evidence that Rg1 could facilitate activation of PI3K/Akt pathway [9, 25]. Rg1 could also mediate phosphorylation of ERK *via* a series of intracellular signal transduction pathways including CaMKIIα and cyclic AMP, which activate the ERK pathway [29]. More recently, Ge *et al.* [21] reported Rg1 protects against 6-hydroxydopamine-induced toxicity in MES23.5 cells *via* both PI3K/Akt and ERK signaling pathways. Interestingly, neuroprotective effects of Rg1 in this experiment seemed to be associated with activation of Akt phosphorylation as well as inhibition of ERK1/2 phosphorylation. This is distinct from a previous study in which phosphorylation of ERK1/2 was promoted by Rg1 [29]. Therefore, further experiments are necessary to clearly elucidate the role Rg1 plays in the ERK signaling pathway.

Taken together, these results propose that signaling pathways like PI3K/Akt and/or ERK are involved in Rg1 regulation of HIF-1α in the setting of neonatal HIBD, and suggest a novel therapeutic mechanism of Rg1 in this condition: Rg1 could increase HIF-1α levels *via* PI3K/Akt and/or ERK signal pathway, regulate its downstream effector molecules such as VEGF, CC3, and EPO, and consequently lead to an increase in angiogenesis, neuronal cell viability, and neurogenesis after neonatal HIBD. These pathways, mediated by Rg1 induction of HIF-1α expression, may serve as a key mechanism involved in brain repair after neonatal HIBD. Thus, administration of Rg1 could be a promising strategy to repair damage in neonatal HIBD. Fig. (**1**) illustrates this mechanism.

## CONCLUSION AND PERSPECTIVES

As for neonatal HIBD, treatment like therapeutic hypothermia has been in use for over half a century and shown some benefit. However, there are no universally accepted drugs with definite neuroprotective effects for clinical application. The metabolism of the neonatal brain is very active, requiring high levels of oxygen consumption. Therefore, the neonatal brain is quite sensitive to oxygen and/or blood nutrient supplements. Inadequate oxygen/blood supply during HIBD causes energy failure, which leads to neuronal cell apoptosis and dysfunction of the CNS [2]. Enhancing the tolerance of CNS cells to hypoxia, reducing cellular apoptosis, and promoting angiogenesis and neurogenesis all become critical to brain repair following neonatal hypoxia/ ischemia.

Modern studies have shown that ginsenoside has favorable effects such as anti-aging and facilitating function in the CNS [8]. Ginsenoside Rg1 has been identified as one of the most active

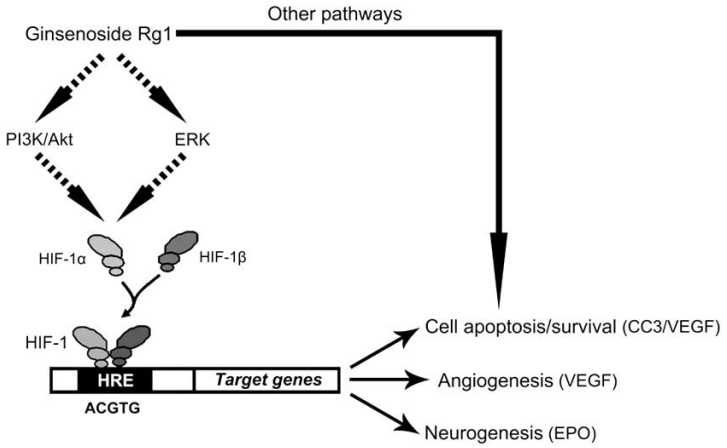


**Fig. (1).** Neuroprotective mechanisms of ginsenoside Rg1: Rg1 could be used to facilitate the process of brain repair, such as cell survival, angiogenesis, and neurogenesis after hypoxia/ischemia brain damage through targeting hypoxia inducible factor-1α (HIF-1α). Cellular signaling pathways such as phosphatidylinositol 3-kinase/Akt (PI3K/Akt) and extracellular signal-regulated kinase (ERK) may participate in this process. CC3, cleaved caspase-3; EPO, erythropoietin; VEGF, vascular endothelial cell growth factor.

ingredients of ginseng, with extensive pharmacological activity, and its protective activity in the CNS is becoming evident.

Ginseng compounds were first demonstrated to play a neuroprotective role in neonatal HIBD by Wang *et al.* [30], who found that ginsenoside could effectively decrease neuronal cell apoptosis and thus increase the survival of neonatal rats with HIBD [30]. Furthermore, Wang *et al.* [28] showed that the reduction of neuronal apoptosis was associated with upregulation of HIF-1α expression. Therefore, HIF-1α related signaling pathways are likely to be involved in cell apoptosis.

Recently, many studies including ours have demonstrated that HIF-1α is likely to play a protective role, such as regulating neuronal cell apoptosis, in HIBD [2]. Although hypoxic stress can activate HIF-1α in the CNS and subsequently trigger brain repair to some extent, the ability of such repair is too limited to achieve a meaningful recovery from HIBD. Therefore, interventions, including Chinese traditional drugs such as *Shenmai injection*, should be tried to increase HIF-1α expression/activity and enhance the neural-protective effects of HIF-1α.

In the last decades, few significant advances of drug development for HIBD treatment have been made. Since Rg1 has been proven to be safe and neuroprotective [31], further studies are needed for its development as a therapeutic agent. These studies should include absorption and pharmacokinetics of Rg1, *in vivo* effects of Rg1 on neonatal HIBD and related mechanisms such as its regulatory effects on ion channel activities and mRNA/protein expression, receptor density and affinity, synthesis and release of neutrotransmitters or neuromodulators, as well as the signal transduction pathways involved. More efforts are expected to be put into such fields in order to determine novel drug targets of Rg1 in the treatment of neonatal HIBD.

## ABBREVIATIONS

| | | |
|---|---|---|
| CC3 | = | Cleaved caspase-3 |
| CNS | = | Central nervous system |
| EPO | = | Erythropoietin |
| ERK | = | Extracellular signal-related protein kinase |
| HIBD | = | Hypoxia ischemia brain damage |
| HIF-1α | = | Hypoxia inducible factor-1α |
| NSCs | = | Neural stem cells |
| PI3K/Akt | = | Phosphatidylinositol 3-kinase/Akt |
| Rg1 | = | Ginsenoside Rg1 |
| VEGF | = | Vascular endothelial cell growth factor |

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## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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