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Original article

Preconditioning and post-treatment with cobalt chloride in rat model of perinatal hypoxic–ischemic encephalopathy

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Abstract

Background: Hypoxia–ischemia (HI)-induced perinatal encephalopathy is a major cause of acute mortality and chronic neurologic morbidities such as cerebral palsy, mental retardation, and epilepsy. As the essential transcription factor for the activation of hypoxia-inducible genes, hypoxia-inducible factor 1 alpha (HIF-1α) plays an important role in the pathophysiological response to the stress of HI brain damage. Whether HIF-1α activation promotes neuroprotection in HI tissues is controversial. *Methods:* The left common carotid artery of rats aged 7 days was ligated under anesthesia. The pups were then exposed to hypoxia in a normobaric chamber filled with 8% oxygen and 92% nitrogen for 2.5 h. In the sham control group, the left common carotid artery was exposed but was not ligated or exposed to hypoxia. To assess the time window for effective treatment, the HIF-1α inducer cobalt chloride ($CoCl_2$) was injected subcutaneously 1 day before surgery, immediately or 1 day after surgery. The brain tissues were harvested from the pups of each groups at 1, 2 and 7 days after insult for HIF-1α protein ant its target genes expression and for investigating the injury. Morris water maze tests were performed at postnatal 7 weeks. *Results:* HIF-1α protein levels and its target genes vascular endothelial growth factor, heme oxygenase-1, and insulin-like growth factor 1 were markedly increased after intraperitoneal injection of $CoCl_2$ (60 mg/kg). The target gene inducible nitric oxide synthase exhibited a biphasic time course. HI caused apoptosis and reduced capillary density, which were ameliorated by $CoCl_2$. Both preconditioning with $CoCl_2$ 24 h before HI and administration of $CoCl_2$ 24 h after HI improved long-term reference memory compared with that in vehicle-injected littermate controls. Administration of $CoCl_2$ immediately after HI did not improve spatial working memory. *Conclusions:* $CoCl_2$ activates HIF-1α and protects against brain damage in vivo. The time of administration could be used to manipulate the activity of HIF-1α pathways and promote recovery.

Keywords: Hypoxia-inducible factor-1α; Cobalt chloride; Hypoxic–ischemic encephalopathy; Preconditioning; Postconditioning; Neuroprotection

1. Introduction

Hypoxic–ischemic encephalopathy (HIE) occurs at a rate of approximately 3–5 per 1000 full-term live births in developed countries and up to 10-fold higher in developing countries [1]. Following hypoxia–ischemia (HI), approximately 45% of newborns die or have permanent cognitive impairments, potentially including cerebral palsy, and there are currently no effective therapies [2].

Hypoxia-inducible factor 1 (HIF-1) is an oxygen-labile protein. Under reduced oxygen tension, the oxygen-regulated subunit HIF-1α accumulates and heterodimerizes with its constitutively expressed subunit HIF-1β,

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leading to binding to the hypoxia response element in gene regulatory regions and increasing the expression of HIF-1α target genes. To date, more than 100 target genes of HIF-1α with varying functions have been identified [3], such as vascular endothelial growth factor (VEGF) [4] , insulin-like growth factor 1 (IGF-1) [5], heme oxygenase-1 (HO-1) [6], and inducible nitric oxide synthase (iNOS) [7]. These genes provide compensation for hypoxia, energy metabolism, erythropoiesis, angiogenesis, vasodilatation, cell survival, apoptosis, and oxidative stress [8]. Thus, HIF-1α has wide ranging roles in the cellular response to hypoxia that are increasingly well established. Evidence suggests that oxygen deprivation and the consequent metabolic compromise in HIE may result from inadequate engagement of adaptive signaling pathways that culminate in HIF-1α activation, while increased expression of HIF-1α dependent genes appears to play an important role in ensuring tight regulation of oxygen homeostasis in the brain to avoid metabolic compromise. However, in addition to these pro-survival effects, evidence suggests that activation of HIF-1α can have pro-death effects. Studies have shown that elevated expression of HIF-1α and increased interaction between HIF-1α and p53 in neonatal HI rats may be an underlying mechanism of the observed neuron damage [2]. Cloning of the rat caspase-3 gene promoter demonstrated the existence of a functional HIF-1 binding element in this promoter [9]. Also, ischemia increased the HIF-1 binding activity of the caspase-3 gene [10]. Acute HIF-1α inhibition may contribute to neuroprotection after HI via preservation of the blood–brain barrier, with subsequent reduction in brain edema and attenuation of neuronal cell death [11]. Thus, HIF-1α regulates the expression of both pro-survival and pro-death genes. The decisive parameters that govern which genes predominate, ultimately deciding the fate of the cell, remain unclear.

HIF-1α activation is controlled by a family of dioxygenases called the HIF prolyl hydroxylases (PHDs) [12]. Using oxygen, iron, and 2-oxoglutarate, these enzymes hydroxylate proline residues in the HIF-1α molecule to allow it to bind to the von Hippel-Lindau-E3 ubiquitin ligase complex and undergo proteasomal degradation. In this scheme, divalent cations such as the Co^{2+} in cobalt chloride ($CoCl_2$) compete with iron and inhibit the activity of PHDs, leading to stabilization of HIF-1α [13]. It has been shown that $CoCl_2$ treatment after left common carotid artery occlusion in neonatal rats is neuroprotective compared with saline treated rats; this effect is mediated through prolongation of the expression of HIF-1α [14]. Thus, $CoCl_2$ can augment the transcriptional activity of HIF-1α and has become an attractive target for therapeutic purposes. However, some studies have indicated that $CoCl_2$ promotes neuron-like PC12 cell damage via upregulation of HIF-1 induced transcriptional activation [15], and how to control the amount and timing of appearance of the functional product of HIF-1α for best effect is less well understood. In the brain, where oxygen homeostasis is crucial, it is important to further understand how to control the activation of HIF-1α for the development novel therapeutic interventions before the dichotomy of HIF-1α activation is explored.

Herein, we investigate the role of $CoCl_2$, a hypoxia "mimic", in the regulation of HIF-1α pathways in the neonatal brain, paying particular attention to the effect of time of administration of the drug on promotion of recovery. A principal hypothesis is that the therapeutic effect of $CoCl_2$ on neuroprotection and repair will facilitate recovery after HI damage.

2. Materials and methods

2.1. Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of Chongqing Medical University of China and were in agreement with Chinese national legislation. Timed pregnant female Sprague Dawley (SD) rats were obtained from the Experimental Animal Centre of Chongqing Medical University and housed in individual cages with the highest standards of care under specified pathogen-free level laboratory conditions. After birth, the pups were housed with their dam under a 12:12 h light–dark cycle, with food and water available ad libitum throughout the study.

2.2. HI brain damage (HIBD) rat model

On 7 days after birth, male and female littermates underwent carotid ligation surgery and cerebral hypoxia using the procedure described by Levine and Rice, with modifications [16]. Briefly, under 2% isoflurane anesthesia, a 0.5 cm ventral midline incision was made and the left common carotid artery was exposed and permanently double-ligated with 4–0 Surgisilk. Age matched sham controls had the carotid artery exposed under the same anesthetic but not ligated or exposed to hypoxia. Following a 30 min recovery period, pups with ligated carotid artery were exposed to hypoxia for 2.5 h (8% oxygen, 92% nitrogen at a flow rate of 6 l/min adjusted with a flow meter) while being maintained at 37 °C. The pups were then allowed to recover in room air and placed with their mother and littermates.

2.3. Compounds and experimental groups

$CoCl_2$ was obtained from Sigma (St. Louis, MO) and dissolved in 0.9% NaCl. For this study, 60 mg/kg $CoCl_2$ was administered subcutaneously [14]. To assess the time