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Chapter

Hypoxic Brain Injury

Zeynep Özözen Ayas, Gülgün Uncu and Demet Özbabalık Adapınar

Abstract

Hypoxic brain injury (HBI) is a clinical condition that results from a decrease in brain blood flow and oxygenation. The damage due to cerebral hypoperfusion is caused by many possible reasons, which leads to severe wide spectrum of clinical presentations. It can be difficult to manage disease process of HBI because the clinical outcomes are poor and treatment options are limited. Neuroprotective trials against different underlying pathophysiological pathways are promising. In spite of all the difficulties, promising signals are obtained in the recent studies. In this article, we aim to provide the details of neurotoxic mechanisms and new interventions for neuroprotection of HBI.

Keywords: hypoxic brain injury, neuronal death, treatment

1. Introduction

Hypoxic brain injury (HBI) is a clinical condition that results from a decrease in brain blood flow and oxygenation. Energy in the brain is mainly derived from oxygen and glucose by 95% oxidative metabolism [1, 2]. About 50% of the energy obtained is used for communication and synaptic activity between neurons, 25% for the passage of ions through the cell membrane and 25% for molecular transport and biosynthesis [3, 4]. Metabolic need increases in seizure and fever but decreases in deep coma and anaesthesia. HBI can be defined as a damage to brain cells due to hypoxia. In HBI, the clinical definition is more complex, indicating hypoxia with many etiologic causes, and broad-spectrum brain injury caused by ischemia with or without reperfusion. Clinic developmental stage of the brain, condition of development of damage, regional weakness, ethology, difficult predictability of treatment and outcomes are heterogeneous due to the differences in accepted guidelines and management standards. Although effective treatment has not yet been found, progress has been made in the prevention of HBI.

2. Hypoxic-ischemic brain injury

Encephalopathy—the neurologic syndrome composed of abnormalities of consciousness, tone and autonomic control is the hallmark of acute HBI [5]. The stage of encephalopathy depends on the timing and severity of the hypoxia. HBI is an important cause of mortality and morbidity in the paediatric age group. Although effective treatment has not been found yet, progress has been made in the prevention of HBI. Advances in conservation have been achieved with neonatal asphyxia

and mild hypothermia in ventricular fibrillation after cardiac arrest in adulthood and early use of thrombolytics after embolic stroke in adults.

3. Cellular mechanisms of neuronal death following HBI

Neurons consistently require a source of metabolic substrates, especially glucose and oxygen. HI brain injury results from intracellular and intracellular processes during and after the imbalance of the presence and consumption of these substrates in the brain. In animal models, neuronal death after HBI occurs in two phases [6]: Immediately after HBI, neurons begin to die rapidly, possibly a cell death process characterised by necrosis, loss of acute plasma membrane integrity and loss of ATP [7]. In the second stage, neurons die from hours to days [8], primarily through apoptosis [9], which is cascade of active, tightly regulated intracellular pathways. Neuropathological evidence of classical neuronal apoptosis after HBI is less pronounced in humans than in animal models [10]. However, there is no doubt that urgent and delayed neuronal deaths are below neurological damage after HBI. New approaches to salvage neurons following HBI have strengthened the existing understanding of HI-induced brain injury mechanisms to specifically target central mechanisms of neuronal death. We will briefly review excitotoxicity, free radical toxicity and inflammation procedures in order to place these treatments in the context of their targeted cellular mechanisms.

3.1 Excitotoxicity

Glutamate is a stimulating neurotransmitter everywhere in the brain. Under pathological conditions, including HI, neuronal receptors for glutamate are overactive due to pathologically high glutamate concentration in the extraneuronal domain. This high concentration occurs as a result of synaptic release of glutamate pathologically, dysfunction of glutamate uptake mechanisms and release of glutamate from the intracellular metabolic pool. Glutamate receptor overactivation results in neuronal death, hence excitotoxicity. Overactivation of the N-methyl-D-aspartate (NMDA), subtype of the glutamate receptor, was highly effective in neuronal death after HI. NMDA receptor overactivation allows intracellular calcium to rise to toxic levels and causes cell death by activating cytotoxic phospholipases, proteases, lipases and endonucleases. Calcium is also absorbed by the mitochondria, causing loss of ATP synthesis, oxidative stress, release of proapoptotic factors and activation of the apoptotic cascade.

3.2 Free radical toxicity

Free radicals are molecules containing one or more unpaired electrons that allow increased intermolecular reactivity. Primary oxygen-free radical superoxide anion is produced in cells (O_2^-). Superoxide is an important intracellular signalling molecule, as is the metabolite hydrogen peroxide (H_2O_2). Together with the highly reactive hydroxyl radical, O_2^- and H_2O_2 are oxygen-derived free radicals present in the cell. Oxidative stress refers to increased levels of these radicals. Oxidative stress contributes to neuron death after HI [11], by breaking down cellular proteins and DNA.

In addition to oxidative stress, increased nitric oxide (NO), nitrogen-free radical, production is a central mechanism of HI-induced neuronal death [12]. Increased NO production is mediated by neuron-specific NO synthase (nNOS) and elevated by HI (and excitotoxicity)-induced intracellular calcium concentrations.

Endothelial NOS (eNOS), a second NO synthase isoform, controls vascular resistance in all organs, including the brain. Maintaining eNOS activity during and after experimental HI improves cerebral blood flow and neuronal survival [13]; therefore, treatments aimed at reducing neuronal NO production should specifically target nNOS and maintain eNOS activity. In addition to its direct effects, NO interacts with O_2^- to form highly reactive and toxic radical peroxynitrite [14]. Peroxynitrite-mediated peroxidation of lipid components of cellular membranes [15] and mitochondrial proteins oxidative modification [16] are important mechanisms of neuronal damage. In particular, lipid peroxidation changes the cellular membrane structure and function that triggers cellular necrosis or apoptosis.

3.3 Inflammation

Improved results in HBI animal models following inflammation inhibition [17] show that inflammation is an important mechanism of HI-induced neuronal death. After HBI, microglia is activated [18], proinflammatory cytokines, e.g. IL-1 and TNF-alpha. In addition, microglia-derived chemokines increase acutely to receive peripheral immune cells into the brain [19]. HBI activates the complementary stage in the brain [20]. Complement activation results in the formation of membrane attack complexes that form pores within the plasma membranes and lead to cell lysis [21]. Therefore, after HBI, a coordinated inflammatory response emerges, which makes a significant contribution to HBI-induced neuron death in the brain.

4. New treatments for HBI

The understanding of the mechanisms of HI-induced neuronal approaches to neuroprotection have shown promise in pre-clinical studies and early clinical trials. Below, we review some of the most promising approaches at different stages of development from early stage research to clinical studies and FDA approval. Since these therapies may address different mechanisms than those mediating hypothermized neuroprotection, these novel therapies also provide additional neuroprotection to those available from hypothermia therapy.

4.1 Erythropoietin

Erythropoietin (EPO) is an endogenous, hypoxia-derived glycoprotein produced in the kidney that has been shown to first regulate haematopoietic function through EPO-specific receptors. [22]. Recombinant EPO (r-EPO), currently approved to increase erythropoietin in anaemia, has also been shown in animal studies where HBI is neuroprotective [23, 24]. Activation of neuronal EPO receptors prevents HBI-induced activation of NMDA receptors and increases expression of anti-apoptotic proteins, potentially reduces excitotoxicity and reduces apoptosis [24, 25]. EPO receptor activation also inhibits HBI-induced stimulation of peroxynitrite (oxidative stress) and inflammatory cytokines, potentially reducing free radical toxicity and inflammation. [25]. EPO receptor expression, which is of particular importance for neonatal HBI, is abundant in the developing mammalian brain [26]. Systemically administered r-EPO after HBI has been shown to cross the blood-brain barrier [27]. In one study, the pharmacokinetics of EPO levels in cerebrospinal fluid in babies treated with EPO after HBI was parallel to that observed in serum [28], suggesting that r-EPO could cross the blood-brain barrier in humans.

4.2 Melatonin

Melatonin is a pineal gland hormone secreted in response to environmental light-dark cycles [29]. Melatonin has multiple cellular effects, two of which directly target known mechanisms of HBI. First, melatonin reduces free radical toxicity, scavenging hydroxyl radical and peroxynitrite by direct electron transfer [30]. Melatonin also reduces O_2^- production in brain slices in vitro following hypoxic ischemic stress [31]. Second, melatonin has anti-inflammatory activity. Thus, after umbilical cord occlusion in fatal sheep, melatonin reduced the production of 8-isoprostanes [32], a potent mediator of HBI-induced inflammation. In addition, melatonin given to rats immediately after focal cerebral ischemia decreased neutrophil migration and macrophage/activated microglial infiltration after 48 hours and decreased only in the ischemic hemisphere [33]. Finally, melatonin reduces the binding of NF-yaB to DNA, resulting in the production of proinflammatory cytokines including interleukin-2, interleukin-6 and tumour necrosis factor alpha [34]. These cellular effects have led to extensive investigation of melatonin as a treatment for hypoxic brain damage.

Short-term assessments of melatonin, infarct size and neurobehavioural outcomes in rats after focal cerebral ischemia are improved [33], suggesting that melatonin treatment may be applicable to global brain ischemia in the newborn. However, short-term improvements may reflect only the temporary inhibition of death-induced procedures without altering the final extent of neuronal death. Finally, melatonin may have a neuroproductive effect in addition to hypothermia. Following induction of global ischemia in newborn pigs, melatonin with hypothermia reduced MR spectroscopic indices of impaired cerebral energy metabolism compared to hypothermia alone [35].

4.3 Allopurinol

Allopurinol is a xanthine oxidase inhibitor that is a source of cytosolic O_2^-, which has attracted attention as a potential neuroprotective agent during HI, especially as it can cross the placenta to produce therapeutic levels in newborns [36]. Animal models including in vivo and in vitro rat models and in vivo sheep models have demonstrated that allopurinol is neuroprotective [37].

4.4 Topiramate

Topiramate is an anti-epileptic drug of interest as a potential neuroprotective agent for brain injury. Topiramate prevents seizures by inhibiting neuronal excitability, including blockade of glutamate receptors [38]. This potential anti-excitotoxicity effect suggests topiramate as a candidate treatment for HBI. Indeed, following carotid artery ligation in the rat, topiramate significantly reduces neuronal death through inhibition of glutamate receptor activity [39], reducing HBI-induced neuronal apoptosis [40]. Of particular interest is the observation that topiramate, when combined with hypothermia, adds neuroprotective effects in animal models. [41].

In the pilot study, topiramate associated with whole-body hypothermia in 27 asphyxia infants did not cause any adverse effects, short-term outcome differences or pathological cerebral magnetic resonance imaging incidence compared to 27 controls [42]. Further extensive clinical studies are needed to assess the efficacy of topiramate in preventing HI injury.

4.5 Xenon

Xenon is a chemically non-reactive gas that is extensively studied as a general anaesthesia in Europe [43, 44], due to its highly favourable safety profile. One of

the activities of xenon is against NMDA receptor activation, which reduces excitotoxicity. This reduced activity results from the xenon glycine block that binds to its regulatory region on the receptor [45]. Following hypoxia or excitotoxicity in cultured murine neurons, increased xenon concentrations significantly increased neuronal survival [46]. In neonatal rats, xenon inhalation improved both histological and functional outcomes 2 months after global HI [47]. Similarly, following global forebrain ischemia in the newborn pig, xenon inhalation proved neuronal survival 72 hours after insult [48]. In particular, in these models, xenon-induced neuroprotection has been found to add to the neuroprotection provided by induced hypothermia.

4.6 nNOS inhibition

The central role of NO in HI-mediated neuronal injury and the presence of specific small molecule inhibitors of nNOS make nNOS inhibition a potentially attractive approach. With the discovery of the toxic role of NOS in HI, early studies of NOS inhibitors have yielded contradictory results since early inhibitors do not have isoform specificity [49]. However, newer, specific nNOS inhibitors may promise more [50]. Prophylactic use of highly specific nNOS inhibitor JI-10 in preterm fetal sheep increased neuronal survival following deep asphyxia [51]. Although initial data for selective nNOS inhibitors are promising, the extent of non-target effects, such as inhibition of eNOS activity and any accompanying reduction in cerebral blood flow, will need to be investigated to initiate clinical trials.

4.7 Pluronic co-polymers

After HBI, the functions of cellular membranes may change due to lipid peroxidation and lipid signalling changes. After severe HI, neuronal plasma membrane dysfunction leads to reduced membrane integrity, infiltration of intracellular components into the extracellular space and necrosis. When HI is not severe enough to induce necrosis, HI-mediated dysfunction of mitochondrial intracellular membranes can trigger apoptosis [52]. Recently, a class of synthetic molecules has been used to address HI-induced dysfunction of injured neuronal membranes in pluronic, in vitro and in vivo. Pluronics, which consist of poly [ethylene oxide] (PEO) and poly [propylene oxide] (PPO) chains, have been arranged in a three-block PEO-PPO-PEO structure. This structure allows the pluronics to interact with the cellular membranes [53, 54] and recovers the integrity of the plasma membrane after injury. Pluronic F-68, a member of Pluronics, has been shown to immediately rescue neurons from death in in vitro HI models by apoptosis blockage [55, 56]. Preliminary evidence also shows that Pluronic F-68, provided to animals for 1 week after HI, significantly improves neuronal survival in the hippocampus, a brain region highly sensitive to global HI, and saves hippocampus behaviour [57]. The novelty of this membrane-targeted approach and the lack of toxicity [58, 59] suggest that targeting membrane dysfunction may be a suitable treatment for future HBI.

4.8 Therapeutic hypothermia

The main mechanism underlying hypothermia in reducing ischemic tissue damage is its effect on metabolism [60]. Oxygen use decreases by 7% almost linearly with each °C reduction below normal [61]. On the other hand, ischemia becomes more tolerable due to the slowdown in metabolism, although a decrease in blood pressure of about 5% per degree has been observed. In animal experiments, the brain volume is approximately 4% less at 25°C compared to 37°C. Here, the main

decreasing cerebral blood flow and volume, the CSF section increases by about 32%. In conclusion, intracranial and venous pressures decrease [62]. In addition, hypothermia reduces the release of excitatory neurotransmitters, such as glutamate and glycine, suppresses free radical toxicity, creates favourable effects on intracellular mediator systems, also reduces intracellular acidosis, inhibits the excretion of ubiquitin, which binds abnormal proteins and facilitates their excretion, anti-apoptotic effects and anti-inflammatory effects and other mechanisms by reducing ischemic neuron damage [63, 64].

5. Conclusion

Hypoxic-ischemic brain injury is a simple imbalance between demand and supply to brain energy. However, cellular mechanisms leading to neuronal death are complex and multifactorial. The overall effectiveness of induced hypothermia is relatively low and the need for mechanism-oriented therapies for HBI is high. Basic research may provide therapeutic targets for translation testing, while defining the underlying mechanisms of HBI-mediated neuronal death. The approaches discussed above target the cellular mechanisms of HBI-mediated neuronal death in many different ways. With ongoing research, one or more of these approaches or their derivatives may ultimately be effective treatments for HBI.

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