Chapter 3
Pathophysiology and Pathology of Neonatal Hypoxic-Ischemic Encephalopathy

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Abstract Hypoxic-ischemic encephalopathy (HIE) is one of the most important diseases in perinatal medicine. The pathophysiology and pathology of HIE are quite unique. The mode of cell death includes necrosis and apoptosis. Necrosis occurs in conditions of primary energy failure following the initial injury. On the other hand, apoptosis occurs days after the initial injury. The damaged area in the brain depends on the mode of injury. Severe and prolonged insults result in diffuse and marked neuronal necrosis. The cerebral cortex-deep nuclear pattern of neuronal injury appears to be related to insults that are less severe and due to partial asphyxia. The deep nuclear-brainstem pattern of injury to the basal ganglia-thalamus-brainstem occurs in infants with total asphyxia.

Keywords Hypoxic-ischemic encephalopathy · Primary energy failure · Secondary energy failure · Total asphyxia · Partial asphyxia

3.1 Introduction

Hypoxia-ischemia in the perinatal period is an important cause of neurological sequelae and associated disabilities in children. In Japan, the incidence of moderate to severe hypoxic-ischemic encephalopathy (HIE) is reported to be 0.37/1000 births. Infants with moderate to severe HIE tend to develop cerebral palsy (CP) [1]. CP is one of the most costly neurologic disabilities (about 100 million JPY/patient) because of its persistence over the life span. In a term infant, the most common mechanism of hypoxic injury is intrauterine asphyxia brought on by circulatory problems, such as clotting of placental arteries, placental abruption, or

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inflammatory processes. These result in perinatal depression leading to diminished exchange of oxygen and carbon dioxide and severe lactic acidosis. In this chapter, the pathophysiology and pathology of HIE are reviewed.

3.2 The Mode of Cell Death in Hypoxic-Ischemic Encephalopathy

There are two fundamental modes of cell death in the nervous system, necrosis and apoptosis. It is now clear that hypoxic-ischemic insults may lead to necrosis and/or apoptosis, which is dependent principally on the severity of the insult and the maturational state of the cell. Certain characteristics readily distinguish these two forms of cell death. Necrotic cell death is characterized by cytoplasmic vacuolation, membrane disintegration, cell rupture, and release of intracellular contents. As a consequence, inflammation and phagocytosis subsequently occur. By contrast, apoptosis is characterized by condensation and margination of chromatin, cell shrinkage, relative preservation of cellular membranes, and cell death without inflammation.

3.3 The Primary and Secondary Energy Failure in Hypoxic-Ischemic Encephalopathy (Fig. 3.1)

Primary energy failure occurs as a result of the initial reduction of cerebral blood flow [2]. The impairment of cerebral blood flow leads to decreases in oxygen and glucose levels, which lead to significantly less adenosine triphosphate (ATP) and increased lactate production [3]. Low ATP levels cause the failure of many of the mechanisms that maintain cell integrity, particularly the sodium/potassium (Na^+/K^+) pumps and mechanisms to maintain low intracellular calcium. When the Na^+/K^+ pumps fail, an excessive influx of Na^+ precipitates massive depolarization of neurons. This leads to the release of glutamate, a prominent excitatory neurotransmitter. The glutamate binds to AMPA/kainate and NMDA glutamate receptors, allowing additional influx of intracellular calcium and sodium [4]. Increased intracellular calcium has significant detrimental effects, which lead to cerebral edema, ischemia, and microvascular damage, with resultant necrosis and/or apoptosis. Most of the effects of primary energy failure lead to cellular necrosis through impaired cellular integrity and disruption of the cytoskeleton and cell membrane.

Necrosis occurs in conditions of very severe hypoxia and ischemia [4]. This causes cells to swell and rupture, leading to cell death. Upon rupture, cellular contents are released, leading to inflammation. When inflammation occurs, there is an influx of microglia to the area, which release inflammatory mediators [5]. Inflammatory mediators can damage the white matter and lead to formation of scar tissue. If the insult is less severe, the cells may recover or progress to apoptosis [4].

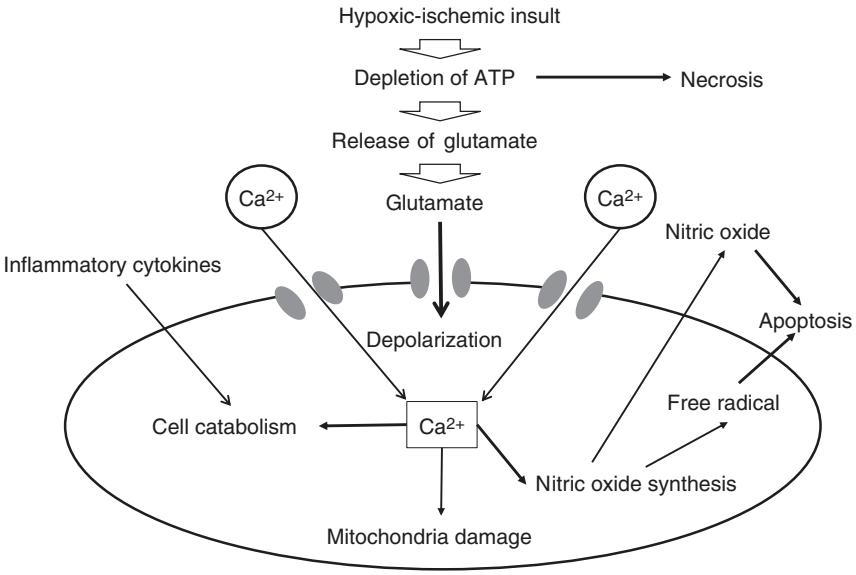

Fig. 3.1 Cell death pathway involved in hypoxic-ischemic brain injury

Apoptosis causes cell shrinkage and general preservation of the cellular membranes with no associated inflammation. Apoptosis can occur days following the initial injury [6]. Both necrosis and apoptosis can lead to decreased brain function.

The extent of primary energy failure contributes to further injury during the secondary energy failure phase. If the hypoxic-ischemic insult is severe, neuronal cell death can occur through necrosis [5]. Once blood flow is restored, there is a brief period of recovery. This brief recovery is the latent period, which is characterized by normal cerebral metabolism. The latent period is thought to vary depending on the extent and severity of the hypoxic-ischemic insult; the more severe the insult, the shorter the latent period is [7]. Currently, the exact timing of the primary energy failure phase, the latent period, and the beginning and ending of the secondary energy failure phase remain unknown [8]. The latent period is considered the optimal timing for therapeutic interventions.

The secondary energy failure phase occurs 6–48 h after the initial injury. The exact mechanisms of secondary energy failure remain unclear but appear to be related to oxidative stress, excitotoxicity, and inflammation. The overproduction of free radicals, which cause damage to neuronal cell membranes and lead to necrosis or apoptosis, causes oxidative stress. Oxidative stress is particularly harmful to the neonatal brain [9] due to low concentrations of antioxidants and a high consumption of oxygen when transitioning from the fetal to neonatal life [10]. Neonates also have high concentrations of unsaturated fatty acids that break down to form more oxygen free radicals. During a hypoxic-ischemic state, protein-bound iron is released, which makes Fe^{2+} available to react with peroxides and form free radicals. The

increased susceptibility to free radical formation and the decreased ability of the neonatal brain to eliminate free radicals lead to damage of neuronal tissue. Excitotoxicity occurs when excessive levels of extracellular neurotransmitters, especially glutamate, overstimulate excitatory receptors. The overstimulation allows additional influx of sodium and calcium into neural cells. Glutamate is used by a variety of neuronal pathways, including hearing, vision, somatosensory function, and learning and memory, which can account for the disruptive effect of HIE on subsequent development. Inflammation is also thought to be important in the development of the HIE-related brain injury [10].

3.4 Pathophysiology of Hypoxic-Ischemic Encephalopathy in a Term Neonate (Fig. 3.2)

A reduction of oxygenation to the fetus leads to bradycardia, which reduces cerebral perfusion pressure as well as oxygenation of neural tissue. The degree to which oxygenation is impaired can also vary. The extent of damage will depend on the degree of impaired oxygenation and the duration of impaired oxygenation; the more severe the degree of impaired oxygenation and the longer the duration of impaired oxygenation, the greater the risk of permanent neurological injury. Additionally, the rapidity of onset of the decrease in flow influences the pattern of injury seen in the brain. In such situations, the fetus faces a sudden profound asphyxia, often termed total asphyxia [11], with a marginally less severe asphyxia which is referred to as near-total asphyxia. This event represents a complete interruption in the supply of oxygen. This total lack of oxygen can be tolerated for only a relatively brief period of time before there is permanent neurological damage and can potentially lead to fetal death. With a sudden decrease in blood flow and oxygen, there is little time for the redistribution of blood flow to protect more mature neurons in the central gray matter of the basal ganglia, thalamus, and brainstem. The relative maturity or immaturity of the developing brain increases or decreases the vulnerability of the tissues to the effects of a decrease in oxygenation and blood flow. Neurons that are mature

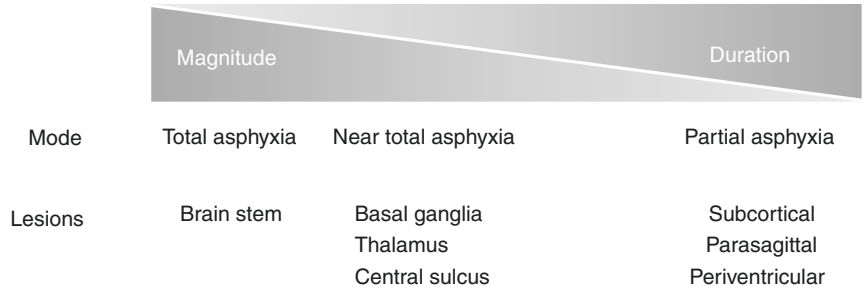

Fig. 3.2 The relationship between mode of injury and lesions

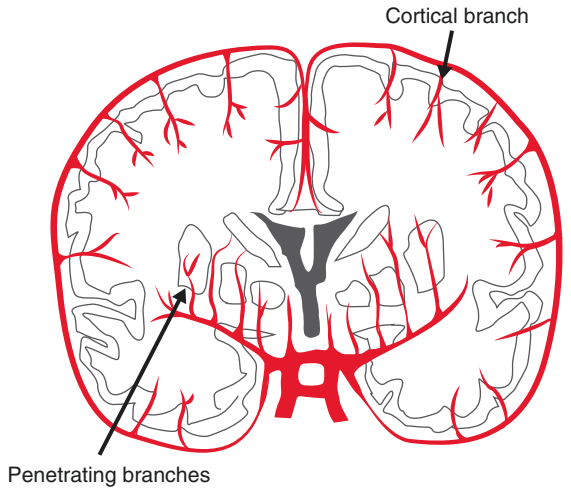

Fig. 3.3 Anatomical characteristics of arteries in cortex/subcortex/white matter

and functional are more vulnerable to a lack of nutrients, whereas cortical areas of the brain that are immature and nonfunctional are accustomed to the relatively hypoxic in utero environment and are less vulnerable [12].

Where the degree of impaired oxygen delivery is less dramatic, the fetus faces partial asphyxia. The fetus is equipped with a number of compensatory strategies that allow it to withstand impaired oxygen gas exchange for hours. The ultimate impact of impaired gas exchange on the fetus will depend on the duration and magnitude of the insult. With a more gradual onset of decreased flow and oxygen, there is time for a relative shift of flow to protect more valuable and vulnerable structures. With a shift in blood flow to the brainstem and central gray matter, the burden of the injury falls on the supratentorial distal portions of the cortex and white matter, in the so-called watershed zones located between the vascular territories (Fig. 3.3), between the anterior and middle cerebral arteries, and between the posterior and middle cerebral arteries (Figs. 3.4 and 3.5).

A fetus may suffer from a partial asphyxia, which is then followed by a total asphyxia. In these circumstances, the fetus is said to have suffered from a "mixed pattern" of asphyxia.

3.4.1 Near-Total Asphyxia

A sudden, marked, or catastrophic decrease in cerebral blood flow or oxygenation to the fetal brain or newborn infant produces near-total asphyxia [11]. The three most common causes for this decrease are placental abruption, cord prolapse, and uterine rupture [13]. Most near-total asphyxia cases are terminal events occurring immediately before delivery. Near-total asphyxia can also occur in a fetus in utero

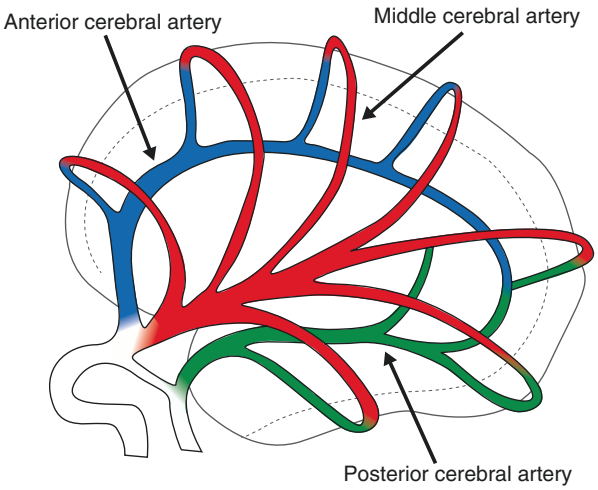

Fig. 3.4 Anatomical characteristics of the three major cerebral arteries

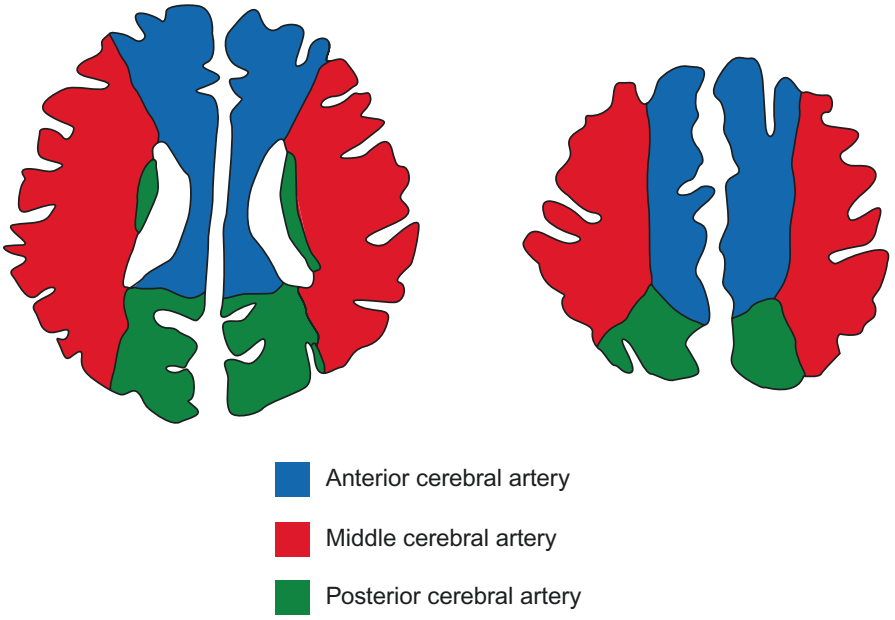

Fig. 3.5 Territories of the major three cerebral arteries

before labor and delivery when the mother experiences a cardiac arrest or cardiovascular collapse as the result of a reaction to an anesthetic or other drugs, has a vasovagal reaction, or goes into shock secondary to trauma, for example, in a motor vehicle accident.

Vulnerability to near-total asphyxia in a term infant is manifested in mature neurons with high metabolic rates that are most sensitive to the deprivation of nutrients [14]. Such regions of vulnerability include the posterior putamina, ventrolateral nucleus of the thalamus, pre- and postcentral gyri (the rolandic cortex region that lies along the central sulcus), and subrolandic white matter that is an area of active myelination at term [12]. In addition, the hippocampi, the superior vermis, and multiple small areas within the brainstem, primarily the cranial nerve nuclei and internal capsules, are also highly sensitive to profound asphyxia [15]. More extensive areas of the basal ganglia can be involved, including other portions of the thalami, the full putamina, globus pallidus, and caudate nuclei. Extensive injuries to the basal ganglia are less common than injuries to the putamen.

3.4.2 Partial Asphyxia

As the fetus comes to full term, the watershed zones in the brain, the areas between vascular territories, shift from the periventricular region toward the cortex and the subcortical white matter (Fig. 3.3). The watershed regions lie anteriorly between the anterior and middle cerebral arteries, primarily in the parasagittal region of the anterior frontal and parietal lobes, and posteriorly between the middle and posterior cerebral arteries in the parasagittal region of the posterior parietal and occipital lobes (Fig. 3.5). There is also an inferior watershed zone in the region of the posterior inferior temporal lobes. Additionally, there is a watershed region between the branches of the vertebral and basilar arteries between the superior cerebellar, posterior inferior cerebellar, and anterior cerebellar arteries in the cerebellum; however, this watershed is rarely found to be involved in a term infant with hypoxic-ischemic brain injury. Watershed territory injuries of the supratentorial brain occur in term fetuses and neonates when there is a reduction in blood flow and oxygenation. The partial asphyxic pattern of injury may occur silently in utero during the last weeks of gestation and become manifest following delivery or at a later point in time. More often it is recognized during the labor and delivery period, when it is associated with events such as cord compression with a nuchal cord, oligohydramnios producing cord compression, or placental insufficiency owing to abnormalities of placental growth and development. Such injuries are most frequently gradual in onset, leading to a progressive but significant reduction in blood flow and oxygenation to the tissue at the end of the vessels in the watershed zones [16]. A series of such events occurring over 1 or more hours results in variable injury to either or both the gray and white matter at the site of the watershed zone. Prolongation of the insult can produce involvement extending beyond the usual watershed region and involving greater portions of the cerebral hemispheres. With further depletion of energy reserves and further diminishing of the fetal heart rate, a pattern of injury may develop that has elements of a partial and near-total asphyxia.

In severe partial asphyxia, the period of reduced blood flow and oxygenation goes on for a sufficiently long time or to a sufficient degree of deprivation of

nutrients that the area of cerebral cortical gray matter and subcortical white matter involvement extends beyond the typical watershed areas of the brain. This involvement produces a more homogeneous and extensive pattern of cortical and subcortical injury with edema and resultant mass effects that tend to involve large portions or all of the cerebral lobes bilaterally.

3.4.3 Mixed Partial Prolonged Asphyxia Leading to Terminal Profound Asphyxia

A mixed form of asphyxic injury occurs when energy substrates are depleted in partial asphyxia, leading to a further insult in the form of a terminal near-total collapse. This injury is seen clinically as a sudden bradycardic event superimposed on a prior more gradual abnormal decline in fetal heart rate. In addition to the damage from the partial asphyxia occurring in, or beyond, the watershed regions, the addition of severe bradycardic events causes injury to the thalamus and putamina with the possibility of hippocampal, vermian, and brainstem injury as well.

3.5 Pathology of Hypoxic-Ischemic Encephalopathy

3.5.1 Selective Neuronal Necrosis

Selective neuronal necrosis is the most common injury response observed after intrapartum hypoxic ischemia. The patterns include diffuse neuronal injury, cerebral cortex-deep nuclear neuronal injury, and deep nuclear-brainstem neuronal injury.

Excitotoxicity is believed to be responsible for the neuronal damage caused by hypoxic ischemia in the developing brain [17]. There is evidence that the neuronal pattern of damage reflects the dysfunction of a set of excitatory neuronal circuits triggering selective neuronal death [18]. Brain injury after transient hypoxic ischemia is an evolving process; transient severe hypoxic ischemia and subsequent reperfusion/reoxygenation lead not only to immediate cell death but trigger complex biochemical events, which result in further delayed neuronal death [19]. However, apoptotic cell death is currently considered to be the main cause of delayed neuronal death based on evidence from hypoxic ischemia in animal models and human infants who subsequently died.

Factors related to the severity and the temporal characteristics of the insult appear to be of particular importance in determining the major pattern of selective neuronal injury in the newborn. Diffuse neuronal injury typically occurs following a very severe, very prolonged insult. The major sites which typically develop diffuse neuronal necrosis in a term infant include the cerebral cortex, hippocampus, deep nuclear structures (the caudate, putamen, and thalamus), and brainstem.

Cerebral-deep nuclear neuronal injury usually occurs following a moderate to severe, prolonged insult and typically consists of injury to the perirolandic cortex/putamen and thalamus. Deep nuclear-brainstem neuronal injury typically follows a severe, abrupt insult. The brainstem, thalamus, and basal ganglia have an active metabolism, and corresponding blood flow is abundant in these areas making them most vulnerable to acute anoxia.

It is postulated that adaptive mechanisms normally operate during asphyxic events. In the most profound and severe insults, blood diverts from the cerebral hemisphere to vital deep nuclear structures. Since deep nuclear structures have high rates of energy use, these nuclei are particularly likely to be injured. In the most prolonged and less severe insults, the diversion of blood to deep nuclear structures occurs, at least to a degree, and the cerebral regions are more likely to be injured after brief, repeated hypoxic-ischemic insults [20, 21].

3.5.2 Parasagittal Cerebral Injury

Parasagittal cerebral injury is a lesion of the cerebral cortex and subcortical white matter with a characteristic distribution over the superomedial aspects of the cerebral convexities [22]. This pattern of injury is characterized by necrosis of the cortex and the immediately adjacent white matter and usually affects the parieto-occipital regions (the posterior watershed) more than the anterior watershed. The precuneus is an area of the brain that lies at the junction of all three major cerebral arteries and is particularly vulnerable to damage in this pattern of injury. At the cellular level, laminar necrosis of cortical pyramidal neurons is typically seen. The likely areas of greatest ischemia relate to parasagittal vascular anatomical factors. Thus, the areas of necrosis in parasagittal cerebral injury are in the border zones between the end fields of the major cerebral arteries (Figs. 3.4 and 3.5). These border zones are the brain regions most susceptible to a fall in cerebral perfusion pressure. The watershed concept is analogous to an irrigation system supplying a series of fields with water and emphasizes the vulnerability of the last fields when the head of pressure falls.

3.6 Conclusion

HIE is one of the most serious birth complications. The hypoxic-ischemic event can be caused by multiple events, but ultimately brain injury occurs because of impaired cerebral blood flow and oxygen delivery to the brain. The phases of injury are categorized as primary and secondary energy failure with the latent period between the phases being the optimal timing for interventions. The majority of treatment strategies target ameliorating the effects of the secondary energy failure. Nowadays, research for new treatments using stem cells against HIE have been conducted.

However, most of them are basic research using model animals. There are a few clinical trials of stem cell therapies against HIE. We must continue to search for ways to prevent and treat the effects of the hypoxic-ischemic event to improve neurological outcomes in infants with HIE.

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