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Rodent Models of Neonatal Brain Oxygen Deprivation

Bruna Petrucelli Arruda, Pamela Pinheiro Martins, Débora Sterzeck Cardoso and Silvia Honda Takada*

Neurogenetics Laboratory, Federal University of ABC, Brazil

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Corresponding author:
Silvia Honda Takada,
Neurogenetics Laboratory, ABC Federal University, Delta block. Federal University of ABC, s/n, 09606-045, São Bernardo do Campo, Brazil,
Email: silvia.honda@ufabc.edu.br

ABSTRACT

The oxygen deprivation at birth leads to hypoxic-ischemic encephalopathy, a serious condition responsible for high mortality index and long-term disabilities in the survivors. There are not known efficient therapeutic strategies to prevent or minimize the sequelae, and there is need of reliable and replicable animal models those represent this condition in its molecular, cellular and behavioral particularities. Here we summarize the rodent models of neonatal oxygen deprivation in order to present the options developed so far in the literature. There is a wide variety of models, each mimicking a different condition, and it is important to be aware that every model has its limitations and none of them represents in totality all the alterations in the human nervous system. The heterogeneity of the condition in humans, in which brain lesions are variable and depend on the severity of the hypoxic insult, its duration and age at which it occurs, makes the development of an ideal animal model much more challenging.

INTRODUCTION

Hypoxic-ischemic encephalopathy is the term used to describe the neurological changes resulting from oxygen deprivation in the neonatal period. It is responsible for 21 to 23% of deaths in term newborns [1] and represents one of the major causes of neonatal brain injury and long-term morbidities. Its incidence is 2-3 / 1000 live births in developed countries and varies between 2.3 and 26.5 / 1000 in developing countries [1,2].

Effective therapeutic strategies to minimize or combat the sequelae of neonatal anoxia are not yet known. The therapeutic hypothermia seems to be neuroprotective in mild hypoxia-ischemia in full-term infants [3], but there are still some conflicting results in preterm babies [4,5], the largest population affected by oxygen deprivation sequelae. Animal models those mimic the hypoxic condition in the immature brain allow a better understanding of the pathophysiological mechanisms of the hypoxic-ischemic encephalopathy and provides input for the development of effective therapeutic strategies to minimize the adverse outcomes.

In the present short review, we intend to summarize the rodent models of neonatal oxygen deprivation.

MODELS

Rice-Vannucci model

The Rice-Vannucci model is an adaptation of the model developed by Levine (1960) in adult rats [6]. It uses P7 rats, whose neurological development corresponds approximately to the 36th week of gestation [7], and involves the unilateral ligation of the left common carotid artery followed by a recovery period of 4 to 8 h, and subsequent exposure to hypoxia, in a chamber saturated with 92% nitrogen for 3.5 hours, at 37°C [8].

In this model, lesions were identified in the ipsilateral cerebral cortex, in the striatum, in the hippocampus, in the thalamus, and in the white matter, the extent of which is related to the duration and severity of the injury [8-12]. Besides, behavioral changes were also observed, such as impaired memory and spatial learning [13,14], attention [14,15], and motor activity [13,16,17]. Although initially developed in rats, the model was also adapted for mice that showed similar neurological and behavioral impairments [18].

Its wide use allows comparison with many publications in the literature; currently, there is ample documentation of the effects of hypoxia-ischemia injury. However, there is a lot of variation in the use of the model between different researchers, such as the latency between ischemia and the hypoxia episode, the time during which the animals are submitted to hypoxia, and the percentage of available oxygen. These variations impair the consistency and reliability of the resulting lesion and, consequently, the replicability of the results.

Hypoxia model

Hypoxia alone is also a model widely used to study neonatal brain injury. In this model, rats at P7 are exposed to 8% oxygen in a hypoxia chamber for 1.5 hours. This model produces histological changes in the subcortex, hippocampus, and lateral ventricle, increased inflammation, impaired neurological development, and short- and long-term behavioral deficits [19]. The hypoxia model alone has also been validated in mice, a species in which it is highly used [20,21].

Although hypoxia alone models can replicate hypoxia mechanisms in humans, the effects depend on the moment of intervention. There is a wide variety as to the age of the animal at the time of the injury, both in rats and mice, and there isn't consensus in the literature about the oxygen pressure, hypoxic exposure time, and body temperature, limiting the replication of results and the study of potential protective agents.

Neonatal anoxia model

The neonatal anoxia model, developed by Dell'Anna et al. (1995), was adapted and validated by Takada et al. [22] to simulate clinical conditions of preterm newborns, most affected by oxygen deprivation. Using rats in P2 and weighing between 6-8 grams, the condition of newborns in the 26th week of gestation is simulated. The pups are placed in a semi-hermetic chamber saturated with 100% nitrogen for 25 minutes, at 37°C ± 1.

This model showed cell death from necrosis and apoptosis in the hippocampus [22], the involvement of Inositol 1,4,5-Triphosphate Receptors (IP3Rs) in the death of hippocampal cells [23], and decreased volume and neurogenesis in that region [24]. Furthermore, adult rats submitted to neonatal anoxia showed impairments in memory and spatial learning [24,25]. Decreased number of cells in the primary somatosensory cortex and the thalamus [26,27] were also observed. Behavioral changes in ontogenetic development and the nociceptive threshold have been reported [26-28].

The advantages of this model are global asphyxia, hypoxemia, and hypercapnia [22], similar to clinical cases in human preterm babies, and a lesser degree of invasiveness when compared to other models. Meanwhile, there are limitations related to the model of neonatal anoxia. Due to its minimal invasiveness and systemic action of the methodology used in the model, as previously mentioned, the changes observed are microscopic. Therefore, macroscopic lesions are not evident when compared to more severe models. Moreover, the relative novelty of this neonatal anoxia model could be a disadvantage, once few studies employed this method, and there is limited comparison of the results obtained by the studies. However, it is an advantage when the unexplored mechanisms of this model were considered as an opportunity for future studies to deepen in, for example, the evidence of white matter injury.

Perinatal asphyxia model

In 1991, Bjelke and colleagues established a model that mimics perinatal asphyxia in rats [29]. When the female rats reach the last day of pregnancy (P21), they are submitted to

hysterectomy and the uterus horns containing the fetuses are removed and immediately submersed to a saline bath at 37°C, where they are maintained for a period of 10 to 22 minutes. Then, neonates are removed from the uterus horns and stimulated to breathe when respiratory recovery is not spontaneous.

As advantages, once the gestation of the dams is kept until the last day, the fetuses are exposed to normal hormonal surges of natural birth, and the oxygen deprivation stimulus in the present model is global, which are analogous to the clinical cases. Besides the high reproducibility, this model also counts with the possibility to adjust the severity of the stimulus based on the variations in the time of submersion, the temperature, and the saline solution. Thus, to promote mild, moderate, or severe asphyxia, uterus horns can be respectively submerged for 10 min, 15 min, or 19 to 22 min [30,32].

If the rodent neurodevelopment is compared to a human, rats from first to the third day of life recapitulate the 23 to 32 weeks of human brain development, when the maturation of the oligodendrocytes, development of the neuroimmunological system, and establishment of the blood-brain barrier is occurring [7]. Thus, once this perinatal asphyxia rodent model is performed in P0 rat neonates, it mimics cases of asphyxia in extreme premature human babies and can affect a wide range of neurodevelopmental events. However, the majority of the studies employing this model are focused on grey matter structures and neurotransmitters systems [29,31,33], and in humans, grey matter injuries are secondary, once the main deficits affect the white matter, as in the cases of periventricular leukomalacia [34]. An important factor that should be considered when this perinatal asphyxia model will be chosen is that dams destined for the hysterectomy are submitted to euthanasia during the procedure. Thus, another female rat that will foster the pups should be considered.

Continuous and intermittent chronic hypoxia models

In 2006, Kanaan and colleagues compared the models of continuous chronic hypoxia and intermittent chronic hypoxia to evaluate two processes that are negatively influenced by the decrease in oxygen, angiogenesis, and myelination. The model consists of using P2 rodents housed with their mothers in isobaric practices. The chambers receive a combination of gases containing nitrogen (N_2), oxygen (O_2), and carbon dioxide (CO_2) regulated to maintain a final concentration of 11% O2 and CO2 < 0.01%. The temperature and humidity are maintained at 22-24°C and 40-50%, respectively. Under these parameters, to mimic continuous chronic hypoxia, the O_2 level is constantly maintained at 11%, while to mimic chronic intermittent hypoxia the O_2 concentration is maintained at 11% for 4 minutes followed by a period of 4 minutes in which this concentration is raised to 21%. [35].

Oxygen deprivation due to continuous chronic hypoxia and intermittent chronic hypoxia characterize different pathological states involving the cardiorespiratory system that can occur in early childhood, for example, asthma, obstructive sleep apnea, and congenital heart disease [36]. However, with the decrease in oxygen, important neurodevelopmental processes are interrupted, such as oligodendrocyte maturation and angiogenesis, as evidenced by Kanaan, contributing to cognitive and behavioral impairment [35].

A limitation found in this model is the maternal exposure to chronic hypoxic treatment, since the dam must be maintained with the nest. Thus, to minimize maternal exposure, there is the possibility of rotating mothers between groups of normoxic and hypoxic offspring, although there is evidence that this rotation is detrimental to the development of the offspring, due to the maternal stress generated by the relay. Mothers may have their behavior altered, influencing the care of their offspring, such as the feeding of their pups. This leads to possible malnutrition and, consequently, to a great bias [37-39].

Prenatal hypoxia

In 2003, Lavrenova and colleagues standardized a model of prenatal hypoxia which affects the period of most active brain formation in rats embryogenesis [40]. This model consists of submitting Wistar female rats on day 14 of pregnancy to a normobaric hypoxic environment for 3 hours. Briefly, in 10 min the oxygen of a 100 L normobaric hypoxia chamber is gradually reduced from 21 to 7%. The dam is kept under this level of oxygen deprivation for 3 h, and the temperature is maintained at 22°C [40].

Offspring submitted to this model of prenatal normobaric hypoxia presented changes in the postnatal ontogenesis, as retardation and delay of innate motor reactions [42], and also deficits in cognition, as a decline of the ability to learn new instrumental reflexes [42], and impairment on the novel object

recognition test [43]. A disruption in the generation and migration of neuroblasts, and consequently decreased in the number of pyramidal cells in the cortex [44] and hippocampus [45] of the offspring were observed. Animals also presented changes in the activity of enzymes important for production and catabolism of Aβ, which could predispose to de Aβ accumulation predisposition with aging [46,47].

An important consideration about this model is that prenatal hypoxia is elicited before the initiation of the myelination in rodents, which occurs from GD18 to P10 [48]. Thus, differently from other models of neonatal oxygen deprivation cited in this article, common lesions on the white matter which match with the periventricular leukomalacia present in clinical cases are not the principal damage caused by this model.

Middle cerebral artery occlusion

The middle cerebral artery occlusion model was originally developed by Koizumi and colleagues in 1986 to mimic ischemic stroke in adult animals since 80% of strokes are caused by thrombosis or embolisms that occur in the middle cerebral artery [49]. In the methodology proposed by Koizumi, the insertion of a monofilament with a silicone tip is used through an incision in the common carotid artery, advancing through the internal carotid artery to the Willis Circle, and stopping at the entrance of the middle cerebral artery [50].

In 1989 the methodology was modified by Longa and colleagues. They also used a monofilament, but it was inserted through an incision in the external carotid artery before being guided by the internal carotid artery [51]. The difference between the Koizumi and Longa methods goes beyond the filament insertion path. In the Koizumi method, occlusion is performed permanently in the common carotid artery, while in the Longa method it is the external carotid artery that is kept permanently closed [52].

Currently, the middle cerebral artery occlusion model is thought to mimic ischemic stroke in neonatal animals due to the increased incidence of cases, with an occurrence of 1/2800 to 1/5000 live births, and leading to the onset of serious sequelae such as cerebral palsy, impaired cognitive development, and epilepsy to those who survive [53-55]. Among the most common causes of neonatal encephalopathy, ischemic stroke encompasses approximately 5-10% followed by hypoxic-ischemic encephalopathy which covers 50-80% of medical complications [56].

Considering that between the 8th and 12th postnatal days the brain of the rodents has a maturation comparable to the brain of a newborn human at term [57], the work developed by Tsuji and colleagues brings interesting results that show that permanent occlusion of the middle cerebral artery in rodents with 12 postnatal days (P12) induces a selective and isolated cortical infarction in the vascular region with corresponding laterality of the artery subjected to occlusion, also reflecting in behavioral changes such as impaired motor control in the rotarod test when compared to animals in the sham group and animals in the hypoxia-ischemia group, and reduced locomotor activity in the open-field test [55].

In 1995, Ashwal and colleagues adapted the permanent occlusion model to a reversible occlusion model in young rats with P14-P18, their results showed that the occurrence of the lesion is dependent on the time of occlusion of the middle cerebral artery. They used occlusion times of 2, 3, and 4 hours followed by 24 hours of reperfusion, these times resulted in cortical infarction of 25, 50, and 80% in animals, respectively [58]. More recently, in 1998, Derugin, Ferriero, and Vexler used the reversible occlusion model with reperfusion 3 hours after the occlusion surgery in 7-day-old neonates. The infarction volume was assessed 24 hours after reperfusion through histology with 2,3,5-Triphenyl-Tetrazolium Chloride Solution (TCC) and evidenced the onset of a focal non-hemorrhagic lesion [59] compatible with findings found in magnetic resonance imaging in children born at term at 10 days of age who suffered an ischemic stroke, which showed a diffuse restricted lesion in the cortex where there was occlusion of the distal middle cerebral artery [60] with areas of less restriction lesion in connected structures, such as the corpus callosum, striatum, and nucleus of the thalamus.

It is important to mention that the middle cerebral artery occlusion model mentioned here has limitations, such as the mortality rate of the animals and the difficulty to be performed in newborn pups under the age of P7 that correspond to the cerebral immaturity observed in premature newborn humans. Thus, new adaptations have been developed in order to introduce studies on the changes caused by ischemic stroke in immature brains.

Influence of sexual dimorphism

Concerning gender differences, the literature points out that boys are more susceptible to oxygen deprivation compared to girls, with a higher mortality rate or sequelae caused by the hypoxic or anoxic event at birth [61.62]. Different models used exhibit variations in cognitive performance and biochemical and cellular changes between males and females [63]. In the HI model, males appear to develop ventriculomegaly and greater tissue loss in the cerebral cortex and hippocampus, while females have the most decrease in the volume of the hemisphere in which the occlusion occurred [64-67]. In addition, Lechner and colleagues demonstrated that hippocampal atrophy after HI in rodents is related to seizure episodes only in males and that although males and females have experienced the loss of positive parvalbumin neurons, treatment with hypothermia has been effective only in females [68].

Females submitted to HI have a greater memory deficit compared to males [69], while males appear to have the largest motor damage [70]. The mechanisms of cell death also differ between the sexes; evidence points out that cell death occurring in the female's brain is mainly linked to the activation of caspase, whereas males show greater susceptibility to oxidative stress [64]. A major impasse in understanding the impact of sexual dysmorphism is probably because most researchers do not prioritize the analysis of sexual dimorphism. Therefore, most studies use only males, and many of other studies do not refer to the sex of the animals used, and others use both sexes as if there was no difference between them.

CONCLUSION

Due to the high incidence of brain injuries caused by the oxygen deprivation at birth, there is an urgent need to develop animal models that mimic the pathophysiology and sequelae of this condition. Despite efforts to develop a complete and efficient rodent model which satisfy all the parameters observed in human babies, there are important limitations in all the current models developed so far. The heterogeneity of the condition in humans, in which brain lesions are variable and depend on the severity of the hypoxic insult, its duration and age at which it occurs, makes the development of an ideal animal model much more challenging.

In this regard, we highlight the need for advances in the search for models that can get closer to what occurs in the human concerning the condition resulting from the insult. Currently, a double-hit model is being studied. In this model, an inflammatory insult (in the mother or neonate itself) and oxygen deprivation are combined. Such model seems promising, because there is ample evidence that maternal inflammation, wich is reflected in the fetus, may predispose to oxygen deprivation at birth [71,72]. Nevertheless, we reinforce the importance and contribution of all these models to describe the different changes resulting from oxygen deprivation, and to the search for neuroprotective agents against these injuries.

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