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Treatments and therapeutic protocols for the recovery of an asphyxiated new-born: A review of pre-clinical and clinical studies in human neonates and in different animal models

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Abstract: The objective of this review is to ascertain the advantages and disadvantages of several treatments and therapeutic protocols that have been used for the prevention and treatment of perinatal asphyxia in human neonates and in different animal models. Perinatal asphyxia is one of the main causes of mortality worldwide and is an important factor in triggering physio-metabolic disorders that result in serious neurological consequences and learning disorders not only in human foetuses and neonates, but also in animals. In recent years, the search for new pharmacological protocols to prevent and reverse physio-metabolic disorders and brain damage derived from perinatal asphyxia has been and continues to be the subject of intense research. Currently, within these pharmacological protocols, therapeutic strategies have been evaluated that use respiratory and hormonal stimulants, as well as hypothermic therapies in combination with other putative neuroprotective agents. Similarly, energy supplements have been evaluated with the objective of preventing perinatal asphyxia and treating new-borns with this condition, and to decrease the incidence of neonatal and foetal deaths associated with it. However, despite these promising advances, this pathology has persisted, since the administration of these therapies in low doses may not exert a neuroprotective effect or, in high doses, can trigger adverse effects (such as reduced cardiac contractility, reduced cerebral blood flow, poor perfusion, sympathetic and neuroendocrine stimulation, and increased blood viscosity) in human foetuses and neonates as well as in different animal models (rats, piglets, sheep and rabbits). Therefore, it is important to determine the minimum effective dose with which these therapies exert a neuroprotective effect, as well as the mode of administration, the duration of therapy, etc. Therefore, until a powerful strategy is found to improve the consequences of suffocation, this topic will continue to be the subject of intensive research in the future.

Keywords: hypoxic-ischemic encephalopathy; neonatal mortality; perinatal asphyxia

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Introduction

Perinatal asphyxia represents one of the main causes of mortality in human neonates, representing 23% of mortality in new-borns worldwide (Ashraf 2017). According to the World Health Organization (WHO), the annual incidence of perinatal asphyxia is approximately 9 million cases, with a mortality of 1.2 million cases (Emechebe et al. 2016). Asphyxia is defined as the inability of a new-born to initiate and maintain spontaneous breathing (Ogunlesi et al. 2013) which can be caused by various maternal or foetal factors such as: prolonged rupture of the membranes, meconium aspiration, multiple births, low birth weight, bad position for birth, placental abruption, increased labour time due to the increase in litter size [i.e., the parturition can be increased from a bit more than 2 h per 12 piglets (Peltoniemi et al. 2020) to 6 h 40 min per 19 piglets born (Yun et al. 2019; Peltoniemi et al. 2020)], and antepartum haemorrhage (Majeed et al. 2007; Aslam et al. 2014). It is a condition that occurs mainly when the gas exchange between the mother and the foetus is altered and is characterised by hypoxaemia, hypercapnia, and ischaemia which cause an immediate redistribution of blood flow to vital organs, therefore, the flow to other tissues is compromised causing a cascade of biochemical changes, leading to neuronal cell death and subsequent brain damage (Nemeth et al. 2016; Barkhuizen et al. 2017). The mortality rate for human neonates who have experienced asphyxia is approximately 45% while 25% of those who survive have changes on physiological and metabolic levels as well as to the central nervous system (CNS), which is why they exhibit neurological disorders that can range from cerebral palsy, encephalopathy, motor disorders, as well as seizures or cognitive disorders of varying severity, such as attention deficit, hyperactivity, mental retardation and neuropsychiatric syndromes (Boskabadi et al. 2015; Capani et al. 2016). Therefore, because asphyxia represents one of the main causes of perinatal mortality worldwide and is an important factor in serious neurological in neonates and foetuses, various experimental studies have been carried out in recent years (Fanos et al. 2014; Jimenez-Bravo et al. 2016; Laptook et al. 2017) in order to create protocols that will facilitate the identification, reduction and prevention of physio-metabolic alterations and brain damage caused by perinatal asphyxia. Hence, the objective of this review is to analyse the advantages and disadvantages of the application of various treatments and therapeutic protocols commonly used for the prevention and treatment of perinatal asphyxia in human neonates and in different animal models that have been used in human medicine.

DEFINITION

During the last 25 years, studies with animal models have facilitated the discovery of pathophysiological mechanisms and new pharmacological protocols and, therefore, have become essential in the search for new strategies and in the development of neuroprotective interventions to treat and prevent alterations of perinatal asphyxia in human neonates (Misbe et al. 2011; Lingam et al. 2016). For example, studies using small animals (mice, rats, and rabbits) to examine neonatal brain injuries have provided informative data (e.g., they have evaluated and defined optimal combinations of different neuroprotective drugs), while studies using larger animals (piglets, sheep and primates) have provided additional information necessary to start clinical trials in humans, as the latter have complex neurological characteristics that are more comparable to humans, such as similar grey/white matter proportioning and substantial cortical folding (Misbe et al. 2011). Likewise, it is important to highlight that the investigations carried out using different animal models have been the basis for developing the practices that are used today as therapeutic strategies to prevent and to treat the effects of perinatal asphyxia in human neonates (Lingam et al. 2016).

The use of animal models in the study of perinatal hypoxia-ischaemia has a history of more than 200 years. Perinatal asphyxia represents one of the main causes of mortality worldwide; not only in human neonates, but also in various animal species, and is the main cause of perinatal mortality of non-infectious origin. In humans it represents 23% of mortality in new-borns worldwide (Lingam et al. 2016; Ashraf 2017). In the case of various animal species, the reported mortality from perinatal asphyxia is 20% in porcine neonates (Muns et al. 2016); 19.5% in foals (Pirrone et al. 2013; Carluccio et al. 2017); 17% to 30% in puppies (Veronesi et al. 2009; Tonnessen et al. 2012; De Cramer et al. 2017);

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from 2.5% to 8.6% in calves (Probo et al. 2012) and 20% in lambs (Robertson et al. 2018). Asphyxia is a condition that occurs when the gas exchange between the mother and the foetus is disturbed and characterised by hypoxemia, hypercapnia, and ischemia, which cause the immediate redistribution of blood flow to vital organs and, therefore, the flow to other tissues is compromised, which causes a cascade of biochemical changes whose events lead to neuronal cell death and brain damage (Nemeth et al. 2016; Barkhuizen et al. 2017).

CLASSIFICATION AND IMPORTANCE OF PERINATAL ASPHYXIA

The reduction in oxygen availability during asphyxiation in human neonates and foetuses and also in other mammals (for example: piglets, lambs, dogs, and foals) is classified into three types: Type 1) Hypoxaemia, which implies a reduction of oxygen in arterial blood without necessarily affecting the functions of the organs and cells (Sanchez-Salcedo et al. 2019a). Type 2) Hypoxia, which results from a significant reduction of oxygen in the inhalation of air and, consequently, in the tissues. Therefore, the oxygen (O_2) supply is inadequate for the oxygen requirements of the tissues and the aerobic metabolism is completed with the anaerobic metabolism to maintain the energy balance (Tsikouras et al. 2017), and Type 3) asphyxiation, in which hypoxia spreads to the central organs and can lead to metabolic acidosis (Sanchez-Salcedo et al. 2019a) because, when hypoxia is prolonged, blood is redistributed from the non-vital organs (liver, kidney) to the brain and anaerobic metabolism produces lactic acid (hence, metabolic acidosis). Since oxygen deficiency is accompanied by acidosis, the energy balance can no longer be maintained which leads to reduction in organ function eventually resulting in permanent tissue damage (Tsikouras et al. 2017).

Pathophysiology of perinatal asphyxia

As a secondary consequence of the hypoxia, ischaemia, and energy failure that are triggered during asphyxia, the new-born may have hypoxic-ischaemic encephalopathy (HIE) (Cullen-Benitez and Salgado-Ruiz 2009; Antonucci et al. 2014). The damage due to HIE is a complex process, beginning with hypoxia and continuing through the recovery period. According to Millan et al. (2018), during this event, there are two main phases in which energy failure occurs, a primary phase corresponds to tissue hypoxia followed by a secondary phase characterised by partial recovery after re-oxygenation/reperfusion (Figure 1). The primary energy failure phase is triggered by a deficit in the supply of oxygen to cells and tissues, which causes a significant decrease in the adenosine triphosphate (ATP) and increased lactate production during this primary energy failure. Due to the above, during this phase, the inactivation of the ATP-dependent ion pumps occurs, which causes an excessive flow of sodium ions in the cytoplasm, cellular inflammation and the inhibition of synaptic recapture of neurotransmitters (Canovas-Ahedo and Alonso-Alconada 2019). Similarly, during this first phase, the lack of ATP hinders the physiological function of the ion pumps and, since the cells fail to maintain the electrochemical gradients, depolarisation occurs due to the influx of sodium ions (Na^+) and Ca^{2+} in the cell, which causes the flow of potassium ions (K^+) (Belov Kirdajova et al. 2020). The elevated extracellular concentration of K^+ causes the opening of L-type voltage-gated Ca^{2+} channels (Luoma et al. 2011; Belov Kirdajova et al. 2020). Ion pumps and exchangers are unable to keep up with the increasing concentrations of Ca^{2+}, which is not pumped out of the cell, and high Ca^{2+} levels trigger the release of glutamate, a major contributor to ischaemia-induced excitotoxicity in neurons and glial cells (Papazian et al. 2018; Belov Kirdajova et al. 2020). Subsequently, the high energy phosphate levels [inorganic phosphocreatine/phosphorus (Pi) and ATP] are seen to be recovered, constituting a latent phase (duration of 6 and 18 hours) (Vannucci et al. 2004). However, this improvement is transient, so this gives way to the secondary energy failure phase, in which many of the pathophysiological mechanisms involved in the development of neonatal brain damage are activated. The secondary energy failure phase occurs 6–48 h after the initial injury (Allen and Brandon 2011). In this phase, the energy failure mechanisms produce oxidative stress (OS), excitotoxicity, inflammation and mitochondrial dysfunction. Increased free radical levels cause damage to the neuronal cell membranes and lead to necrosis or apoptosis. OS is particularly damaging to the neonatal brain due to low antioxidant concentrations and

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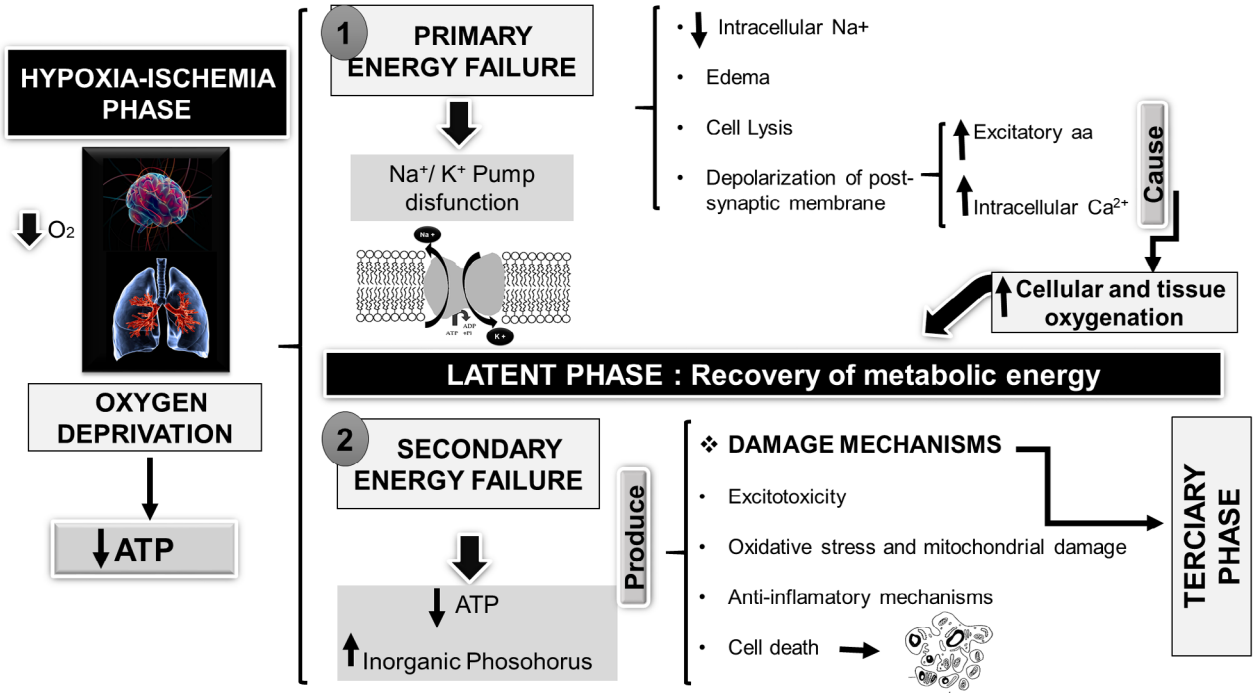

Figure 1. Principal mechanisms that are triggered when neonates undergo a process of perinatal asphyxiation
Adapted from "Combined therapy in neonatal hypoxic-ischaemic encephalopathy", by Canovas-Ahedo and Alonso-Alconada (2019). According to Canovas-Ahedo and Alonso-Alconada (2019), at first the neonate or foetus experiences an ischaemic hypoxic injury, due to the fact that there is a deficit in the supply of oxygen in the cells and tissues, which causes a primary energy failure. Subsequently, the high energy phosphate levels seem to be recovered, constituting the latent phase. However, this improvement is only temporary and gives way to the secondary phase, when many of the pathophysiological mechanisms involved in the development of brain damage in new-borns come into play, highlighting the excitotoxicity, massive entry of Ca^{2+} into the cell, oxidative stress, inflammatory reaction and, in most cases, cell death due to necrosis or apoptosis (Millan et al. 2018). Last of all, the tertiary phase is characterised by the persistence of brain damage in weeks, months or even years after the initial hypoxic-ischaemic insult (Canovas-Ahedo and Alonso-Alconada 2019)
aa = amino acids; ATP = adenosine triphosphate; Ca^{2+} = calcium ion; K^+ = potassium ion; Na^+ = sodium ion

high oxygen consumption during the transition from foetal to neonatal life (Millan et al. 2018). On the other hand, there is a later tertiary phase that is characterised by the persistence of brain damage weeks, months and even years after the hypoxic-ischaemic attack (Canovas-Ahedo and Alonso-Alconada 2019).

Main consequences of perinatal asphyxia

There are different pathophysiological mechanisms that explain hypoxic ischaemic encephalopathy, all linked to each other: a) excitotoxicity, b) intracellular calcium accumulation, c) free radicals, d) nitric oxide, e) cytokines and, f) cell death (Cullen- Benitez and Salgado-Ruiz 2009) (Figure 1). Excitotoxicity is triggered by the extracellular accumulation of excitatory amino acids, mainly glutamate which is the most abundant excitatory amino acid (aa) that causes the receptors that open the sodium channels (which generate cellular oedemas) and calcium to be stimulated. Therefore, due to excitotoxicity, intracellular calcium accumulates in cells (Cullen-Benitez and Salgado-Ruiz 2009; Antonucci et al. 2014) and causes the activation of lipases, phospholipases, proteases and endonucleases, which are enzymes involved in the degradation of cells (Antonucci et al. 2014). Mainly, proteases and endonucleases destroy the cytoskeleton and cellular DNA, respectively. In addition, in view of the fact that the cellular damage caused by hypoxia decreases the antioxidant enzymes (superoxide dismutase and catalase), the DNA and the cell membrane suffer damage, causing the release of free radicals, which, when combined with

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nitric oxide (NO) cause the formation of oxynitrite radicals (ONOO), which further damage the cell membrane (Cullen-Benitez and Salgado-Ruiz 2009). In both human and sheep neonates, plasma concentrations of these NO metabolite (NOx) concentrations fall > 50% within minutes after birth, raising the possibility that the circulating NOx plays a role in maintaining low foetal vascular resistance and in the cardiovascular transition at birth (Blood et al. 2020). Due to the damage caused by the ischaemic hypoxia injury in the general structure of the central nervous system, the glial cells produce cytokines. It is important to note that cytokines are classified as pro-inflammatory (IL-lb, TNFα, IL-6, IL-8) and anti-inflammatory (IL-4, IL-10, TGFα). In the case of asphyxiated neonates, a high concentration of proinflammatory cytokines (specifically IL-6 and IL-8) occurs in the cerebrospinal fluid, therefore, the magnitude of the brain damage is directly related to the concentration of these cytokines (Cullen-Benitez and Salgado-Ruiz 2009; Antonucci et al. 2014). Likewise, it is important to note that after an ischaemic hypoxia injury, neuronal death can occur due to necrosis or apoptosis. Necrosis is caused by a severe injury and occurs within minutes: depletion of cellular energy and loss of membrane integrity result in the leakage of the cytoplasmic contents and a subsequent inflammatory reaction. Conversely, apoptosis is a highly controlled, energy-requiring process that takes more time to develop, and leads to cell death programmed or caused by the same organism. Apoptosis is the dominant form of cell death after less severe brain injuries and in the later phases of the injury process. However, it is noteworthy that both forms of cell death coexist (Antonucci et al. 2014). Additionally, apoptosis has been found more prominent in the immature brain compared to the mature brain (Zhu et al. 2004).

However, despite the fact that the brain is the system most affected by hypoxia that occurs in the asphyxia process, other effects can also be observed, such as peripheral haemodynamic changes and multi-organ dysfunction. The selection of the best animal models that mimic human perinatal asphyxia are essential to generate more effective therapies that allow the prevention, intervention and recovery of human infants with asphyxia. Currently, the most effective therapies that have been generated for the prevention and treatment of perinatal asphyxia are mainly based on the restoration of oxygen and acting synergistically with therapeutic hypothermia, while other therapies are focused on providing energy to the new-born (Oei et al. 2018).

Treatment and therapy of perinatal asphyxia

OXYGEN TREATMENT

Sufficient oxygenation is essential for providing the energy required for normal organ function, growth and development in the neonate. This is because aerobic metabolism is a more efficient system of extracting energy from metabolic substrates than anaerobic metabolism. For this reason, oxygen therapy aims to increase the supply of oxygen to the tissues by using the transport capacity of the arterial blood to the maximum (Walsh and Smallwood 2017; Oei et al. 2018). For this, the amount of oxygen in the inspired gas must be such that its partial pressure in the alveolus reaches sufficient levels to completely saturate the haemoglobin (Hayes et al. 2019). Oxygen is necessary for the adequate metabolism of carbohydrates and the production of ATP (Walsh and Smallwood 2017; Oei et al. 2018). When oxygen levels do not meet the requirements of bodily functions, tissue hypoxia occurs (Walsh and Smallwood 2017). This hypoxia may cause a series of problems, such as localised vasodilation, pulmonary vasoconstriction, metabolic acidosis, tissue necrosis, an increased risk of kernicterus, and impairment of surfactant production, causing brain damage (Walsh and Smallwood 2017; Solevag et al. 2019a). From a pathophysiological standpoint, a complex process takes place at the cellular and tissue level during the development of new-born brain damage in the absence of oxygen (Solevag et al. 2019a). Initially, the lesion is triggered by a deficit in the supply of oxygen to the cells and tissues, causing a primary energy insufficiency. Subsequently, high energy phosphate levels recover transiently (the latent phase) that is followed by a secondary phase, in which many of the pathophysiological mechanisms involved in the development of neonatal brain damage ensue (i.e., excitotoxicity, massive influx of Ca^{2+}, oxidative and nitrosative stress, inflammation). This leads to cell death by necrosis or apoptosis. Eventually, a tertiary phase occurs, characterised by the per-

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sistence of brain damage for months and even years after the hypoxic-ischemic event (Cardinali 2019).

On the other hand, it is important to note that in neonatal animals, such as lambs, piglets, mice and in human neonates, artificial ventilation with oxygen therapy is one of the treatments that has been shown to have benefits for apnoea caused by perinatal asphyxia, since it significantly reduces the risk of perinatal mortality and severe ischemic hypoxic encephalopathy in infants with asphyxia (Saugstad et al. 2008). Similarly, this therapy causes the rapid reversal of hypoxaemia and prevents hypo- and hyperoxygenation of the brain (Rawat et al. 2016; Solevag et al. 2019b). Because the oxygen delivery to the brain depends on the cerebral haemodynamics, concentration of the inhaled oxygen and blood oxygen content (Solevag et al. 2019b). However, despite the fact that oxygen therapy has been shown to have positive effects on the neonate and foetus, it can have also harmful effects, since preterm infants have a poor antioxidant defence system and are, therefore, prone to oxygen toxicity (Torres-Cuevas et al. 2016). In addition, O_2 use in the delivery room is associated with potential adverse effects; hyperoxia slows cerebral blood flow, brief periods of 100% O_2 cause long-term reductions in the cerebral blood flow. High concentrations of O_2 lead to generation of oxygen free radicals, which have a role in reperfusion/reoxygenation injury after asphyxia especially to oxyregulator tissues such as in the myocardium (Garcia-Hidalgo et al. 2018). In this regard, studies carried out by Solberg et al. (2012) in neonatal pigs (12 h to 36 h) that were resuscitated with oxygen at 21, 40 and 100% for 30 min, it was observed that the piglets that were treated with higher percentages of oxygen (40 and 100%) exhibited levels of neuroprostanes and neurofurans significantly higher than the piglets that were treated with a lower percentage of oxygen (21%). The above suggests that the higher the percentage of supplementary oxygen used, the higher the levels of lipid peroxidation that occur in the cerebral cortex. Consequently, even intermediate oxygen concentrations, such as 40%, have the potential to harm the brain. Similarly, in a study by Markus et al. (2007) in near-term foetal sheep (gestational age: 133–137 days), resuscitation with 100% O_2 was found to be associated with activation of proinflammation in the brain 2 h after birth. Likewise, another study by Lakshminrusimha et al. (2011) using a lamb model of in-utero asphyxia showed that resuscitation with 100% oxygen induces oxidative stress and causes the pulmonary arteries to exhibit increased contractility similar to that of non-suffocated lambs. However, despite this finding, it is important to note that this increase in contractility is not due to increased oxygen absorption by the lung and does not lead to better systemic oxygen extraction. Therefore, resuscitation with 100% oxygen did not cause any advantage in the correction of pulmonary haemodynamics. In the same line of research, another study carried out on seven-day old neonatal rats showed that the administration of 100% oxygen after an ischemic hypoxic injury causes an increase in the brain injury and also an accumulation of nitrotyrosine, which it is a marker of oxidative stress (Dalen et al. 2012). Thus, previous studies indicate that brain damage may be aggravated by the uncontrolled use of oxygen during resuscitation of the new-born after perinatal asphyxia.

Despite the aforementioned, research in animal models with asphyxia has shown that resuscitation with 21% oxygen concentrations decreases mortality and is adequate to induce pulmonary vasodilation and the return of spontaneous circulation (Rawat et al. 2016). This is because individual responses to oxygen therapy vary greatly, depending on the particular cause of hypoxia and the degree of impairment. Hypoxia caused by hypoventilation and ventilation/perfusion anomalies associated with pulmonary disease will be most responsive to oxygen therapy (Walsh and Smallwood 2017). In this regard, clinical studies have shown that infants resuscitated with 21% oxygen concentrations exhibit faster recovery because they have a significantly higher heart rate and Apgar score compared to those infants resuscitated with oxygen at 100% (Saugstad et al. 2005). This is mainly due to the fact that low oxygen concentrations cause the earlier recovery of the mitochondrial function (decrease of Krebs cycle intermediaries) (Solberg et al. 2010). Likewise, the above agrees with another study carried out by Fanos et al. (2014) in piglets, in which it was shown that resuscitation with 21% oxygen seems to be associated with optimal cellular function and maintenance, while lower oxygen concentrations (18%) and higher (100%), were related to an increase in glucose and the elimination of free radicals, respectively (Fanos et al. 2014). Related to the above, a study by Murgia et al. (2013)

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points out that when pig neonates are resuscitated with low oxygen concentrations (21%), they have a faster recovery time compared to those resuscitated with high oxygen concentrations (100%) (33.7 ± 21.04 min and 57 ± 44 min, respectively). Undoubtedly, oxygen administration is one of the treatments used in the resuscitation of infants with asphyxia that must be adjusted to avoid excessive or inadequate dosing. Given that when oxygen levels do not meet the requirements of the bodily functions, tissue hypoxia occurs (Walsh and Smallwood et al. 2017).

CAFFEINE TREATMENT

Various studies have verified the efficacy of methylxanthines (aminophylline, theophylline, caffeine) for the treatment of hypoxia and apnoea in preterm infants. In these times, especially, it has been observed that of these methylxanthines, caffeine has shown more advantages over theophylline and is considered to be the most benign of the methylxanthines due to its wide therapeutic index, its higher therapeutic ratio, its more reliable enteral absorption and its longer half-life (Orozco-Gregorio et al. 2011). Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid with a central analeptic effect that functions by increasing the muscle performance of the respiratory system and bronchodilation (Menozzi et al. 2015). It acts by blocking adenosine receptors including A1, A2a, A2b, and A3 expressed mainly in the brain, lungs, heart, fat tissue, testicles, and in the retina (Deliktas et al. 2019). This effect is mainly due to the fact that caffeine and adenosine have similar molecular structures; the former has the potential to occupy adenosine receptor sites (especially A1 and A2A), thus blocking the regulatory effects of the latter (Dobson and Hunt 2013). By antagonising adenosine, which has generalised inhibitory functions, the effect of caffeine is broadly stimulatory. In this way, and due to the properties of the endogenous modulator of intercellular signalling, adenosine decreases the cell excitability and, as a result, can produce respiratory depression, since some of its receptors (A1) mediate the inhibition of adenylate cyclase, whereas others (A2) can stimulate that same substance (Orozco-Gregorio et al. 2011; Dobson and Hunt 2013). Apart from breathing stimulation, caffeine, at therapeutic concentrations, reduces oxidative damage by increasing enzymatic/non-enzymatic antioxidants in the tissues (Abreu et al. 2011), and inhibits activation of a proinflammatory cytokine cascade (Tunc et al. 2013; Koroglu et al. 2014). This is mainly because, in high concentrations (20 to 50 times more than necessary to block adenosine receptors), caffeine can act through a second mechanism of action, in which it inhibits cyclic nucleotide phosphodiesterase (PDE), which catalyse the degradation of adenosine monophosphate (AMP) and cyclical guanosine monophosphate (GMP) up to the forms 5'-AMP and 5'-GMP, respectively (Orozco-Gregorio et al. 2011; Menozzi et al. 2015). The inhibition of the aforementioned PDEs leads to the accumulation of AMP and cyclic GMP and, with this, the signalling transduction is intensified across the nervous routes in which they intervene. For this reason, PDE can intensify the activity of endogenous neurotransmitters and increase the ionic permeability of neuronal membranes in an asphyxia process (Orozco-Gregorio et al. 2011). Due to having a chemical structure that allows it to cross the placenta, caffeine can potentially be used to treat foetuses *in utero*, providing protection against hypoxia during parturition (Carrillo and Benitez 2000). This is particularly helpful in polytocous species, such as pigs, and new-born humans with asphyxia. Regarding this, studies carried out in veterinary medicine have verified that the administration of caffeine in pregnant sows (days before their probable farrowing date) increases the respiratory capacity of piglets, increasing their adaptation to the extrauterine environment [partial pressure of oxygen (pO_2) levels in pig neonates from mothers treated with Caffeine: 19.10 ± 0.82 mmHg vs. Control: 14.49 ± 1.42 mmHg] (Sanchez-Salcedo et al. 2019b). In addition, caffeine has been shown not only to have an effect on foetuses before delivery, but also when administered directly to newborns. This has been demonstrated in veterinary medicine, as studies by Orozco-Gregorio et al. (2010) and Orozco-Gregorio et al. (2012) point out that the administration of caffeine orally or subcutaneously in porcine neonates with intrapartum asphyxia causes a decrease in the blood lactate levels with a consequent increase in the pH.

On the other hand, it has been shown that caffeine presents differences in its response according to the sex of the new-born (Ballot et al. 2015; McDonald et al. 2018). This may be due to the fact

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that physiologically, compared with females, males have: less foetal breathing movements *in utero*; delayed lung development (Ballot et al. 2015), surfactant production and sodium transport efficiency prior to 32 weeks' gestational age; larger lungs with lower peak flow rates; longer latencies to achieve SpO_2 > 90% after birth; and deficient antioxidant status. Despite the use of antenatal steroids to promote lung development, intrinsic sex differences confer increased vulnerability in males, who traditionally require more supplemental oxygen, ventilatory support and surfactant use than preterm females (McDonald et al. 2018). In an investigation carried out by Kouchi et al. (2017) in neonatal rats (1 and 12 days of age), it was shown that females exhibit a reduction in the frequency of apnoea and a lower hypercapnia response compared to males. These observations indicate that caffeine has gender specific effects in new-born rats that vary with the modality of administration (acute vs. chronic), the level of O_2 (normoxia vs. hypoxia), or hypercapnia. Studies in human neonates have found that caffeine administered to preterm neonates with a birth weight of < 1 200 g, results in a lower incidence of bradycardia, cyanosis (Caffeine: 19.2% vs. Control: 61.5%) and apnoea (Caffeine: 15.4% vs. Control: 61.5%). It seems that the preventative effects of caffeine on apnoea become apparent by using the drug in very premature infants (Armanian et al. 2016). However, the benefits found with the use of caffeine may have some limitations, such as in the case of porcine neonates, their administration is contraindicated in piglets with weights ≤ 1 000 g (Orozco-Gregorio et al. 2012), while in the case of human new-borns (≤ 30 weeks gestational age) it was found that, when caffeine is administered in high doses (80 mg/kg i.v.) in the first 24 h of life, there is a greater incidence of cerebellar haemorrhage, hypertonicity and the subsequent alterations in early motor performance (McPherson et al. 2015). Therefore, it is important to note that, currently, the American College of Obstetricians and Gynecologists considers that, in the case of pregnant women, should be administered doses of caffeine of 200 mg daily, because although there is no evidence to suggest that caffeine increases the risk of congenital malformations, some studies have found that high caffeine intake during pregnancy (more than 400 mg per day in pregnant women) may be associated with a lower birth weight due to the restriction of intrauterine growth and even a higher risk of miscarriage (Bracken et al. 2003; Chen et al. 2016; Evans et al. 2021). On the other hand, in the case of the treatment of hypoxic ischaemic injuries in human neonates, caffeine is administered at a loading dose of 20 mg/kg, followed by 5 mg/kg to 10 mg/kg per day of caffeine via enteral or parenteral methods (Evans et al. 2021), since, according to Moschino et al. (2020), toxic levels are reached when doses > 40–50 mg/l are administered. Despite several studies exploring the best minimally invasive and cost-effective methods to control caffeine therapeutic ranges in clinical practice, few have attempted to develop a pharmacokinetic model to adjust the caffeine dose (Koch et al. 2017; Gentle et al. 2018; Moschino et al. 2020), for example, in 2017 Koch et al. (2017) began to develop models of simulation of caffeine concentrations and proposed the need to adjust maintenance doses according to the lifetime, for example, in the second week of life, it was proposed to administer doses of 6 mg/kg/day; in week 3 and 4 of life, it was proposed to administer doses of 7 mg/kg/day and in week 5 and 8, it was proposed to increase the dose and to administer 8 mg/kg/day. However, more pharmacokinetic studies are needed in relation to both clinical outcomes (apnoea episodes, extubation failure, respiratory morbidity in the first year of life) and adverse events (tachycardia, hypoglycaemia, seizures, weight loss, neurodevelopment at 2 and 5 years of age) to identify the appropriate dose of the drug (Moschino et al. 2020).

THERAPY WITH THERAPEUTIC HYPOTHERMIA

Definition

At the present time, therapeutic hypothermia (TH) has become the standard treatment for new-borns suffering neonatal hypoxic-ischaemic encephalopathy after perinatal asphyxia (Thoresen et al. 2013). The neuroprotection mechanisms of therapeutic hypothermia are multifactorial. The most important appear to be suppression of excitotoxicity, decreasing oxidative stress, and inflammation modulation of intracellular signalling and programmed cell death (Rodriguez-Fanjul et al. 2017). However, despite the fact that several randomised controlled trials have shown that TH

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reduces mortality and improves the neurodevelopmental outcome among survivors (Gluckman et al. 2005; Shankaran et al. 2014), this therapy does not provide complete neuroprotection (Rodriguez-Fanjul et al. 2017). This treatment starts as soon as possible after birth in order to prevent the secondary deterioration in the energy metabolism and the initiation of the excito-oxidative injury cascade (Brekke et al. 2017). According to Thoresen et al. (2013), the neuroprotective effect of hypothermic therapy has been shown to be within the first 6 h after hypoxia-ischaemia, as the effectiveness of TH decreases with delay. Its neuroprotective effect is mainly related to the reduction of the cerebral metabolism, because, during hypothermia, the cerebral metabolism of oxygen and glucose is reduced with a temperature decrease of 1 °C leading to a 5.3% reduction in the brain energy utilisation rate (Dehaes et al. 2014; Brekke et al. 2017). Consequently, the levels of high energy phosphates, including phosphocreatine and ATP, are better preserved (Brekke et al. 2017), and the lactate concentrations are reduced (Wisnowski et al. 2016). Brekke et al. (2017) point out that one of the mechanisms in which reduced energy utilisation can provide neuroprotection that might be associated with the lower levels of excitatory amino acids (glutamate, aspartate, glycine) observed during hypothermia since excitatory neurotransmission is an energy demanding process. Also, the lowest levels of excitatory amino acids during hypothermia are themselves neuroprotective, because glutamate excitotoxicity is one of the main factors contributing to ischaemic hypoxic brain injury. Similarly, Wood and Thoresen (2015) point out that therapeutic hypothermia causes a linear reduction in both the heart rate and cardiac output and metabolic rate. Therefore, as the temperature drops, the reduced metabolism leads to a decrease in the production of CO_2. This, together with the increased solubility of CO_2 in the blood, results in a drop in pCO_2, with subsequent effects on the cerebral blood flow. Therefore, when the metabolic rate decreases in line with the central temperature, pCO_2 falls by 3–4% when human neonates show a decrease of 1 °C. Due to the above, therapeutic hypothermia exerts a neuroprotective effect in human neonates who experience ischaemic hypoxia in the asphyxia process. However, despite these positive effects that have been observed in hypothermic therapies, it is important to note that hypothermia can also have adverse effects, such as reduced cardiac contractility, reduced cerebral blood flow, poor perfusion, sympathetic and neuroendocrine stimulation, and increased blood viscosity (Alonso-Alconada et al. 2015; Wood and Thoresen 2015). Therefore, it is likely that an effective temperature range exists below which hypothermic neuroprotection is lost. This therapeutic temperature range may be influenced by the severity, delay in the onset and duration of the cooling, and other factors, such as the peripheral immune response (Alonso-Alconada et al. 2015).

On the other hand, it is important to note that therapeutic hypothermia mainly consists of two different cooling methods: 1) Selective cranial cooling or 2) Total body cooling. The basis of the first cooling method is based on the fact that the brain of the new-born produces 70% of the total body heat, therefore, the cooling of the brain tries to minimise the possible adverse effects derived from the systemic cooling. Whereas, the second method is based on the fact that each time the neonate experiences a reduction of 1 °C in its core temperature, there is a reduction in the metabolic rates of the brain and of the whole body (5–8%) (Sanchez-Illana et al. 2017). In both methods, treatment with therapeutic hypothermia can be divided into three phases: 1) Cooling induction, 2) Maintain stability, and 3) Rewarming.

Phases

Phase 1. Induction: The objective of this phase is to achieve a temperature below 34 °C before 6 h of life. Although the optimal cooling rate is unknown, experimental studies indicate that hypothermia is more beneficial the sooner it starts (Tapia-Velasco 2015). For example, studies carried out on short-term foetal sheep biomodels indicate that when brain hypothermia is induced 5.5 h after ischaemia (before the onset of post-ischaemic seizures), hypothermia is partially protective and associated with moderate secondary microgliosis (Gunn et al. 1998; Gunn and Thoresen 2015). However, despite the above, a study by Laptook et al. (2017) in human neonates with a gestational age of 36 weeks and a postnatal age of 6 h to 24 h, demonstrated that when therapeutic hypothermia begins 6 h to 24 h after birth, the probability that death or disability in hypothermic infants was at least 1% less than in non-cooled infants than

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was 71% this using a Bayesian analysis with a neutral prior and making a comparison with those infants who are not undergoing hypothermic therapy.

According to Garcia-Alix and Alarcon-Allen (2013), induction can be carried out in a simple and inexpensive way with ice-cooled serums (30–40 ml/kg of 0.9% NaCl by a peripheral intravenous route), such traditional ice packs placed on the groin, armpits, and around the neck and head. Today, however, this method is less common, since more modern cooling systems have been developed such as: the Ecotherm-Servo mattress, Criticool vests (Curewrap) and Arctic Sun hydrogel patches that are used for total body cooling. Likewise, there are other cooling systems such as the Cool-Cap that is used for selective cranial cooling. All these systems have been developed in order to achieve faster induction and to facilitate a more reliable maintenance of the temperature.

Phase 2. Maintenance: The objective of this phase is to rigorously control the central temperature, since fluctuations in the central temperature (rectal or oesophageal) must be avoided or be small (maximum 0.2 and 0.5 °C, respectively), because temperature elevations cause neonates to have a greater probability of exhibiting disability in childhood (Gunn and Thoresen 2015; Tapia-Velasco 2015). During this phase, neonates who are treated with total body hypothermia exhibit an average temperature of 33 °C to 34 °C, while, in the case of those neonates who are treated with cranial hypothermia, they maintain an average temperature of 34 °C to 35 °C (Garcia-Alix and Alarcon-Allen 2013). In both methods, the temperature is maintained for 72 h, because when hypothermia is maintained for a longer period, neonates exhibit negative effects. For example: in a study by Shankaran et al. (2014) in term human neonates with moderate to severe hypoxic ischaemic encephalopathy, it was shown that when therapeutic hypothermia is carried out for 120 h at 32 °C, neonates have a greater probability of dying, compared to when they are treated with a standard therapeutic hypothermia – duration of 72 h at 33.5 °C (17 vs. 7%, respectively). Similarly, this same study showed that a longer cooling duration (120 h) is associated with higher arrhythmia and anuria, while deeper cooling (32 °C) causes neonates to have a greater need of inhaled nitric oxide therapy and require more days of oxygen use. In another study by Alonso-Alconada et al. (2015), term piglets that experienced an ischaemic hypoxia lesion, cooling at 35 °C and 33.5 °C was found to cause a reduction in the delayed cell death and microglial activation in most brain regions. Therefore, a protective pattern was observed, depending on the U-shaped temperature pattern in delayed cell death in the periventricular white matter, caudate nucleus, putamen, hippocampus, and thalamus. Likewise, in another study carried out on foetal sheep (from 118 days to 124 days of gestation) that experienced an ischaemic hypoxic lesion and that were subjected to therapeutic hypothermia for 72 and 120 h, it was determined that when hypothermia is maintained for 72 h, greater neuronal survival in the cortex and dentate gyrus was exhibited compared to when therapeutic hypothermia is maintained for 120 h (Davidson et al. 2015a). On the other hand, studies carried out by Zhu et al. (2004) reported that neonatal rats with an ischaemic hypoxia lesion experienced a reduction in apoptotic cell death when hypothermia is maintained for 72 hours. Similarly, a study of seven-day-old rats that experienced asphyxia found that, when hypothermic therapies cause a greater reduction in body temperature for 6 h (starting immediately after hypoxia-ischemia), neonates experience better recovery neurobehavioral after 1- and 6-weeks survival (Gunn et al. 2017).

Phase 3. Rewarming: During this phase, slow and controlled heating occurs (with temperature increments of 0.1 °C to 0.2 °C per hour), reaching normothermia within a minimum period of 6 h to 8 hours. It is important to note that this phase must occur gradually, since rapid heating carries the risk of hypotension and seizures (Garcia-Alix and Alarcon-Allen 2013). Likewise, hyperthermia should be avoided in the following hours, since it worsens the neurotoxic cascade and the neurological prognosis (Wyatt et al. 2007; Laptook et al. 2008).

COMBINED TREATMENTS WITH THERAPEUTIC HYPOTHERMIA

Given the evidence discussed above that current clinical hypothermia protocols are reasonably close to optimal, the other key strategy to improve neuroprotection in asphyxiated infants would be to combine known effective hypothermia protocols with other putative neuroprotective agents (Davidson et al. 2015b). Therefore, recent research has fo-

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cused on the development of therapies that can be used in combination with hypothermia, attempting to function at different levels of protection.

Combined treatments with therapeutic hypothermia and allopurinol

During hypoxia, the brain is not only damaged by direct ischaemia, but also via the production of free oxygen radicals after reoxygenation and reperfusion. Allopurinol, a xanthine-oxidase inhibitor, inhibits the conversion of hypoxanthine and uric acid, thereby limiting the production of free oxygen radicals (Klumper et al. 2018). Likewise, it prevents the degradation of adenosine and preserves the integrity of the *N*-methyl-D-aspartate (NMDA) receptor, so it can reduce brain injury from ischaemic hypoxia through various mechanisms of action that are independent of the effect of therapeutic hypothermia in the metabolism of cellular energy (Rodriguez-Fanjul et al. 2017). At high concentrations (40 mg/kg), allopurinol scavenges free radicals such as hydroxyl, chelates free iron, and inhibits lipid peroxidation and the heat shock factor expression (Arteaga et al. 2017a; Klumper et al. 2018). Therefore, hypothermic allopurinol therapy appears to be effective in reducing mortality (18% died with allopurinol vs. 55% of the controls) and morbidity (27% with allopurinol vs. 55% controls) in neonates with perinatal asphyxia (Amin et al. 2017).

The first reports of the neuroprotective effects of allopurinol in animal models were published in the early 1990s. Subsequently, it was tested in humans and beneficial effects were observed with respect to free radical formation, cerebral perfusion, and electrical brain activity, without toxic side effects. Most importantly, these benefits were observed over the long-term in asphyxiated neonates with moderate ischaemic hypoxia (Rodriguez-Fanjul et al. 2017). For example, administration of 40 mg/kg/day (3 days) within 2 h after birth has been shown to improve the neurological development in neonates at 12 months of age or older in asphyxiated human infants (Gunes et al. 2007). Similarly, the intravenous administration of this amount of allopurinol within 4 h after birth in neonates with severe ischaemic hypoxic encephalopathy has no toxic effects, decreases the serum free radical levels, and improves the cerebral blood flow (McNally and Soul 2019). With respect to animal models, the administration of allopurinol in asphyxiated porcine neonates, has been shown to have positive effects on the brain energy status and cytotoxic oedemas (Kaandorp et al. 2010). In addition, in a study evaluating a hypoxic-ischaemic animal model in ten-day-old rats that underwent combined hypothermia plus allopurinol therapy after experiencing moderate hypoxic ischemic injury, it was found that this treatment decreased the incidence of heart attack and improves the residual brain volumes at 72 hours. Furthermore, the benefit of allopurinol was shown to be gender dependent as females treated with allopurinol and allopurinol plus therapeutic hypothermia showed a decrease in apoptosis markers compared to males (Rodriguez-Fanjul et al. 2017).

Despite these positive results, Kaandorp et al. (2010) point out that when the asphyxia has been too severe, the brain damage inflicted can no longer be reversed, so the use of allopurinol during labour in the case of suspected foetal hypoxia, provides the opportunity to start the treatment earlier, thereby limiting the amount of hypoxia reperfusion injury and improving the neurological outcome. This effect of allopurinol during labour is due to allopurinol being able to cross the placenta and quickly reach the appropriate concentration in the newborn. In fact, studies by Boda et al. (1999) verified this effect in porcine and human neonates. They demonstrated that the administration of 30 mg/kg of allopurinol (Milurit®, Egis) in pregnant sows days before the probable date of farrowing, causes the porcine neonates born to sows treated with allopurinol to present high enough allopurinol levels to ensure the effective inhibition of the xanthine oxidase activity. Likewise, in the mentioned study of human neonates, a strong positive correlation (r = 0.87) between the measured allopurinol values was demonstrated in the maternal blood and in the umbilical blood of the infant. A similarly a strong positive correlation of the uric acid levels was also observed in the maternal and umbilical blood samples (r = 0.875) (Boda et al. 1999; Schober et al. 2018). As a result of the above, administration of allopurinol in combination with therapeutic hypothermia in asphyxiated neonates appears to be a treatment with beneficial effects, so a clinical trial called "Effect of allopurinol for Hypoxic-ischemic Brain Injury on Neurocognitive Outcome", is currently underway. ClinicalTrials.gov Identifier: NCT03162653 (Rüdiger Mario, PhD, University Hospital Tuebingen, Germany.

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Research in process). The recruitment of human infants (gestational age ≥ 36 weeks and birth weight ≥ 2 500 g, with severe perinatal asphyxia and potentially progressive encephalopathy) began in April 2018 and ended in April 2020. The last patient to come out (after follow-up) is expected in April 2022 (Maiwald et al. 2019). This trial attempts to evaluate the therapeutic potential of allopurinol during the first minutes of life (Canovas-Ahedo and Alonso-Alconada 2019). In fact, recent studies have shown that this trial (NCT03162653) in human new-borns who received an initial allopurinol dose of 20 mg/kg for a period of 10 min within 45 min after birth and/or hypothermic therapy (with a second allopurinol dose of 10 mg/kg – 12 h after the initial dose) indicate that the dosing regimen applied in the NCT03162653 trial leads to the inhibition of xanthine oxidase in new-borns treated with or without therapeutic hypothermia. These findings may form the basis for further treatment optimisation and individualisation of the allopurinol dose in new-borns with hypoxic ischaemic encephalopathy (Chu et al. 2021).

Combined treatments with therapeutic hypothermia and erythropoietin (EPO)

Erythropoietin (EPO) is a glycoprotein cytokine that is synthesised during the foetal period by the liver and postnatally by the kidney and the brain, and that acts as a growth factor and neuroprotective agent (Cardinali 2019). EPO is a cytokine with pleiotropic functions including erythropoiesis, modulation of the inflammatory and immune responses, vasogenic and pro-angiogenic functions, and effects on the brain development and repair (Wu and Gonzalez 2015). Primarily, EPO exerts its anti-inflammatory action after binding to its receptor (EPOR) which is expressed on the neuron membranes, astrocytes, oligodendrocytes, and microglial cells (van Bel and Groenendaal 2016). In the setting of hypoxia-ischaemia, EPOR expression is rapidly upregulated, with EPO production increasing only if significant hypoxia is prolonged. If EPO is available to bind to the upregulated receptor, cell survival is promoted, but in the absence of EPO, the pathway of programmed cell death predominates (McAdams and Juul 2016). Therefore, the beneficial effect of erythropoietin on ischaemic hypoxic encephalopathy is based on its action on the specific receptors present in the neurons and glia, capable of developing a powerful antiapoptotic activity (favouring gene transcription of antiapoptotic Bcl-2 and Bcl-xL), as well as having anti-inflammatory and antioxidant properties. In addition to its neuroprotective effect, EPO is capable of promoting long-term reparative processes, such as angiogenesis, oligodendrogenesis, and neurogenesis (Canovas-Ahedo and Alonso-Alconada 2019; Cardinali 2019).

Numerous experimental studies in different neonatal species (for example: rodents, sheep, and non-human primates) who sustained ischaemic hypoxic brain injury after experiencing asphyxia, have provided consistent evidence that the exogenous EPO administration results in a 34% to 70% improvement in the neuroprotection of the newborn [exerts a neuroprotective effect before, during and after an ischaemic hypoxic event is experienced (Rangarajan and Juul 2014)] (Wu and Gonzalez 2015). EPO has both early [(i.e., before a hypoxic ischaemic event or in the first 72 h of a new-born's life): before a hypoxic event, it prevents brain injury and, after a hypoxic event, it inhibits apoptosis-like cell death in the ipsilateral ischaemic cortex] and late beneficial effects [(i.e., 5 weeks to 12 weeks after experiencing a hypoxic ischaemic event): significantly improves long-term spatial memory deficits; facilitates recovery of sensorimotor function] (Wen et al. 2006). Early benefits include anti-apoptotic and anti-inflammatory effects, while later effects include increased neurogenesis, oligodendrogenesis, and angiogenesis, all of which contribute to tissue remodelling after hypoxic-ischaemia (McAdams and Juul 2016). The effects of erythropoietin are mainly dose dependent, since multiple dose administrations are found to be more efficient in the neuroprotection of the neonate compared to single doses. For example, in neonatal rats, it has been observed that a single dose of EPO (5 000 U/kg) administered immediately after the neonates experience an ischaemic hypoxic lesion, significantly preserves the hemispheric brain volume and improves the behavioural function in the short term, however, this effect does not persist in animals when they reach adulthood. Whereas, in contrast when they receive multiple doses (three doses of EPO over a period of 1 000 U/kg), they exhibit a long-term improvement in the brain volume and cognitive function (Larpthaveesarp et al. 2017). Studies carried out on human neonates with moderate and severe hypoxic ischemic encephalopathy

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indicate that the administration of multiple doses of EPO (300 U/kg to 500 U/kg, every second day for 2 weeks) causes infants to be less likely to die or have moderate or severe disability at 18 months of age compared to infants who were not treated with EPO (Zhu et al. 2009). Similarly, studies carried out by Elmahdy et al. (2010) on human neonates with the same lesions, demonstrated that the administration of multiple EPO doses (five daily doses of 2 500 U/kg) reduces the oxidative stress biomarkers (i.e., NO) after 2 weeks and improves the neurodevelopment at 6 months of age compared to human neonates with hypoxic ischaemic encephalopathy who were not treated with EPO.

In actuality, therapeutic hypothermia (TH) is the only proven effective intervention for treatment of ischaemic hypoxic injuries in much of the world (i.e., the USA, Europe, and Australia). It significantly reduces the mortality by 8.8% and severe morbidity by 15.4% (Lutz et al. 2020). For this reason, the use of EPO in the treatment of asphyxiated neonates experiencing ischaemic hypoxic lesions has been more recently studied in combination with therapeutic hypothermia (Wu and Gonzalez 2015). In this regard, a study carried out on non-human primates who exhibited ischaemic hypoxic lesions after experiencing asphyxia and who underwent combined therapies of therapeutic hypothermia plus EPO showed that the application of these types of therapies improves the motor and cognitive responses (recognition memory). Likewise, this treatment causes a decrease in the probability of mortality and disability (Traudt et al. 2013). In the same line of research, a study by Fanos et al. (2014) in seven-day-old rats, in which the clinical aspects of asphyxia were simulated, showed that the addition of EPO to hypothermia had a neuroprotective effect in the neonates. Additionally, a 26% improvement in the sensorimotor function was observed in males. This effect of EPO in males may be due to the fact that during this period of development, testosterone acts as a masculinisation factor of the male genital tract and the reproductive behaviour, influencing the development of related anatomical elements in the peripheral tissues, but also in the brain and spinal nuclei of males (Ballot et al. 2015; McDonald et al. 2018). In particular, as circulating steroids easily cross the blood-brain barrier, perinatal testosterone affects the development of brain areas favouring cell death or survival in different sexually dimorphic neural groups that are involved in the male sexual behaviour (sexual dimorphic nucleus of hypothalamic preoptic area) and in the neural control of the male genital tract (spinal nucleus of the bulbocavernosus muscle and dorsolateral nucleus in the lumbar spinal cord). Because of the above, it is believed that the specific effects of EPO could be regulated by the perinatal secretion of testosterone (Ballot et al. 2015). Regarding human neonates, a study carried out by Frymoyer et al. (2017) demonstrated that when neonates are administered combined hypothermia + EPO therapies (dose of 1 000 U/kg every 24 h for the first 2 days), they manage to exhibit EPO levels associated with neuroprotection in animal models [i.e., area under the curve during the first 48 h of treatment (AUC 48 h) 140 000 mU × h/ml]. Despite the fact that previous studies have shown beneficial effects in hypothermic therapies with EPO, it is important to note that EPO is ineffective at promoting neuroprotection at very low doses (i.e., 250 U/kg) and may cause harm at very high doses (i.e., 5 000 U/ml) (Weber et al. 2005; Wu and Gonzalez 2015). The significance and severity of the adverse effects related with EPO administration are controversial. For example, in premature infants, EPO administration has been associated with the risk of neutropenia (Pet and Juul 2014; Wu and Gonzalez 2015). However, this has not been confirmed in later studies of erythropoietic or neuroprotective dosing (Pet and Juul 2014). There has also been concern about the increased risk of retinopathy of prematurity in preterm infants treated with EPO, particularly early in life, but the data are conflicting, and this is not relevant to term infants (Xu et al. 2014). Due to the above, three phase III clinical trials are currently underway with the goal of evaluating the safety and efficacy of high EPO doses (1 000 U/kg) in 840 neonates in combination with therapeutic hypothermia (Cardinali 2019): 1) Erythropoietin for Hypoxic Ischaemic Encephalopathy in New-borns (PAEAN Trial), ClinicalTrials.gov Identifier: NCT03079167 [Principal Investigator: University of Sydney, Australia (Helen Liley, MD)]; 2) High-dose Erythropoietin for Asphyxia and Encephalopathy, ClinicalTrials.gov Identifier: NCT02811263 [Principal Investigator: University of California, USA (Yvonne Wu, MD)]; 3) Erythropoietinin Management of Neonatal Hypoxic Ischemic Encephalopathy, ClinicalTrials.gov Identifier: NCT03163589 [Principal Investigator: Assiut

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University, Egypt (Samia Mohamed, MD) (Wassink et al. 2019)]. The primary objective of the first two trials is the reduction of death or disability at two years, while the third will evaluate these parameters after one year. It will be necessary to wait for the results of these and other studies to determine the minimum effective dose, its mode of administration, the duration of therapy, etc. (Canovas-Ahedo and Alonso-Alconada 2019).

Combined treatments with therapeutic hypothermia and melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) is a neurohormone primarily produced by the pineal gland at night. It provides circadian and seasonal rhythm cues and is a potent antioxidant (Gitto et al. 2009). To a lesser extent, melatonin is also produced outside of the pineal gland within the skin, retina, ovaries and gastrointestinal tract and is readily found within the cerebrospinal fluid (CSF), bone marrow and bile (Aridas et al. 2018). Primarily, melatonin was validated as a neuroprotective agent in asphyxiated neonates, as it was observed to improve energy metabolism and immune function, as well as anti-inflammatory, anti-apoptotic and anti-cytotoxic effects (Cardinali 2019). Furthermore, it was validated as a neuroprotector because it has antioxidant properties that help directly eliminate the production of reactive oxygen species (ROS) (Aridas et al. 2018) and therefore induces a protective effect against oxidative stress by intervening in the positive regulation of antioxidant enzymes such as glutathione peroxidase, glutathione reductase and superoxide dismutase (Cardinali 2019). An additional benefit of melatonin is that it prevents apoptotic cell death via stabilisation of the mitochondrial function and prevents the release of proapoptotic proteins in response to hypoxic ischaemic injury (Aridas et al. 2018; Cardinali 2019). Therefore, the potential use melatonin for use as a therapy for neonates who experienced hypoxic ischaemic injury due to asphyxia, is based on its remarkable antioxidant and anti-inflammatory power, as well as its ability to traverse the blood-brain barrier and reach the central nervous system (Canovas-Ahedo and Alonso-Alconada 2019). For example, application of melatonin in low doses (0.1 mg/kg/day) and administered for seven days before the delivery due date has been shown to protect mice foetuses against the effects of asphyxia at birth. This is mainly due to the fact that melatonin acted as a powerful free radical inhibitor and as an indirect antioxidant (Wu and Gonzalez 2015). Along these same lines, in another study carried out by Signorini et al. (2009) where the formation of oxidative damage mediators and the effect of melatonin was evaluated in a model of hypoxic-ischemic encephalopathy in seven-day-old rats. It was observed that neonates are treated with melatonin show a significant reduction in levels of free iron, F(2)-isoprostanes and F(4)-neuroprostanes.

Therefore, in this study, melatonin showed a reduction in the oxidative damage. Similarly, in another study in neonatal rats with hypoxic ischaemic injury, it was observed that the administration of melatonin had an antioxidant effect, since it stimulated the gene expression and activated superoxide dismutase (SOD), catalase, G6PD and glutathione reductase (Wu and Gonzalez 2015). The effect of melatonin has also been evident in other animal models. For example, in a model of porcine neonates that experienced perinatal asphyxiation, the neuroprotective effects of the combination of melatonin (5 mg/kg/h over 6 h) with hypothermic therapies (33.5 °C from 2 h to 26 h after resuscitation), it was found that the combination of these two therapies results in a significant reduction in the ischaemic hypoxic injury, since despite the fact that neonates experienced ischaemic hypoxic injury during perinatal asphyxia, they showed higher levels of ATP and a reduction in the level of the prototypic cytotoxic microglial activation marker CD86 (Robertson et al. 2013). In the present study, the brain ATP levels are protected by this treatment, because melatonin prevents increased lactate/*N*-acetyl aspartate in the reperfusion phase (increased lactate/*N*-acetyl aspartate as an indicator of compromised or damaged mitochondria) (Robertson et al. 2013; Aly et al. 2015). At the clinical level, in a study published by Aly et al. (2015) in asphyctic human neonates; it was demonstrated that the application of hypothermic therapies combined with the administration of melatonin (5 doses of 10 mg/kg/day/oral route) cause a reduction in the serum levels of superoxide dismutase and nitric oxide, which shows that the application of the combination of both therapies effectively reduces oxidative stress and improves survival with favourable results of neurological development at 6 months of age. In order to assess the safety, pharmacokinetics, dosage and effectiveness of the

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use of melatonin in combination with hypothermia in human neonates, a recent study by Balduini et al. (2019) has shown that hypothermia does not affect the pharmacokinetics of this neurohormone, and high blood values can be reached (area under the curve in 24 h = 4.35 μg/ml × h] with lower doses (0.5 mg/kg) than used in experimental models. Moreover, the MELPRO study [ClinicalTrials.gov Identifier: NCT03806816. Principal Investigator: University Hospital of Ferrara, Italy (Anna Tarocco, MD)] is currently in the process of recruiting patients, with the goal of evaluating 100 neonates in order to analyse the application of melatonin in clinical practice (Canovas-Ahedo and Alonso-Alconada 2019).

Combined treatments with therapeutic hypothermia and N-acetylcysteine

N-acetylcysteine (NAC) is a promising antioxidant therapy that impacts many pathways of injury and has established neuroprotective effects in animal models that experienced an ischaemic hypoxic injury in the suffocation process (Nie et al. 2016). Mainly, it has been observed that NAC is a scavenger of oxygen radicals and a precursor of glutathione. Likewise, it can eliminate the production of ROS, restore glutathione levels, mitigate the redox potential, reduce apoptotic cell death and diminish both inflammatory cytokines and inducible nitric oxide synthase in an adult rat stroke model (Arteaga et al. 2017a). It has low toxicity and it is able to cross the placenta and the blood-brain barrier. In most experiments evaluating the neuroprotective effects of NAC, repeated administration has been used (Hobson et al. 2013), or the administration of NAC in combination with therapeutic hypothermia. In this regard, studies carried out by Lee et al. (2008) and by Liu et al. (2010) in porcine neonates that experienced hypoxic ischaemic injury found that the administration of NAC reduces the production of hydrogen peroxide (H_2O_2) (thus decreasing the oxidative stress) and lactate, as well as improving the cerebral blood flow and also lowers caspase-3 levels, all of which play a role in cell death and are considered a specific marker for apoptosis. On the other hand, in a study carried out in neonatal rats, it was observed that when NAC (50 mg/kg) is administered in combination with hypothermia (30 ± 0.5 °C, induced immediately after hypoxic injury and maintained for 2 h], resulted in an increase in the level of myelin [Myelin Basic Protein (MBP) and Proteolipid protein (PLP)] and a significant reduction in the volumes of cerebral infarction (NAC + Hypothermia: 21.5 ± 3.84 mm^3 vs. Control: 240.85 ± 4.08 mm^3] (Jatana et al. 2006). Similarly, in another study by Nie et al. (2016) evaluating the efficacy of NAC in combination with hypothermia in neonatal rats experiencing severe ischaemic hypoxic injury, it was shown that the administration of NAC (50 mg/kg/d) 1 h after initiation of hypothermia, the inducible nitric oxide synthetase (iNOS) expression and caspase 3 activation in the injured hemisphere significantly decreased. However, only females treated with hypothermia + NAC (50 mg/kg/d) showed improvement in infarct volumes in the short term. For this reason, the authors point out that antioxidants may provide insufficient neuroprotection in males in the short term, while long-term therapy may be suitable for both sexes. In view of the above, NAC seems to be an attractive therapy given its multifactorial approach and its previous use in other disease models with minimal side effects. However, more studies are needed to investigate the combinational therapy and its use in human subjects, as there is still no consensus on an optimal dosage or therapy duration (Hobson et al. 2013).

Combined treatments with therapeutic hypothermia and noble gases

Noble gases, such as xenon and argon, have demonstrated neuroprotective effects in models that experienced ischaemic hypoxic injury during perinatal asphyxiation. It has been shown that xenon doubles the level of neuroprotection after moderate hypoxic-ischaemic brain injury in new-born animal models when combined with hypothermia (Sabir et al. 2016) due to its ability to decrease the excitotoxicity after hypoxic ischaemic injury, by modulating the glutamatergic NMDA receptors (Canovas-Ahedo and Alonso-Alconada 2019). In a study of neonatal rats experiencing ischaemic hypoxia during asphyxiation, the application of combined xenon therapies (20%) with therapeutic hypothermia was shown (35 °C for 90 min initiated 4 h after hypoxia-ischaemia) to cause a synergistic increase in the neuroprotection, particularly through the intrinsic apoptotic pathway. Importantly, combined xenon and cooling is effective even when the xenon administration is delayed for some hours (Ma et al. 2005).

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Similarly, in another study carried out in a model of porcine neonates with perinatal asphyxia, the application of inhaled xenon at 50% in combination with hypothermia (33.5 °C) was evaluated and a reduction in the cerebral abnormalities in the biomarkers of magnetic resonance spectroscopy and in cell death markers in some brain regions was seen ($P < 0.05$) (Faulkner et al. 2011). However, a recent study by Sabir et al. (2016) in neonatal rats with severe ischaemic hypoxic brain injury and who underwent combined xenon therapy (50% inhalation) with hypothermia (32 °C), indicates that immediate therapeutic hypothermia with or without additional 50% inhaled xenon, does not provide neuroprotection one week after severe hypoxic ischemic brain injury. Moreover, studies carried out by Azzopardi et al. (2016) in human neonates indicate that the combined therapies of therapeutic hypothermia and xenon did not show significant differences between groups.

In light of the above, because some studies have not shown significant effects in the combination of these therapies and with the aim of deepening some of the variables that may have influenced the treatment with this noble gas, a clinical trial is currently underway in phase II called "Xenon and Cooling Therapy in Babies at High Risk of Brain Injury Following Poor Condition at Birth: A Randomised Pilot Outcomes Study (CoolXenon3 Study), ClinicalTrials.gov Identifier: NCT02071394 [Principal Investigator: University of Bristol, UK (Marianne Thoresen, PhD) (Wassink et al. 2019)]. On the other hand, there have been no studies carried out in human neonates using argon to date. However, a study by Broad et al. (2016) in a model of porcine neonates who presented an ischaemic hypoxic lesion and who underwent combined therapies of argon (45–50% inhaled argon) and therapeutic hypothermia (33 °C) from 2 h to 26 h, showed that the combination of both therapies improves the results obtained by hypothermia, with respect to the *N*-acetyl aspartate/lactate biomarker, which is related to an increase in the brain death values and the development of neurological issues in the new-born. According to Canovas-Ahedo and Alonso-Alconada (2019), these promising results, together with their higher bioavailability and lower cost compared to xenon, make argon a molecule with high translational potential in the treatment of ischaemic hypoxic injuries in asphyxiated neonates.

THERAPY FOCUSED ON METABOLIC ENERGY LEVELS

Primarily, when perinatal asphyxia occurs, the absence of oxygen causes the aerobic metabolism to change to anaerobic, causing neonates and foetuses to exhibit a significant (60%) decrease in ATP levels and cause an accumulation in lactate (Souza et al. 2012). According to Logica et al. (2016), when energy levels decrease as a secondary consequence of oxygen and glucose deprivation, foetuses and neonates experience hypoxic-ischaemic injury that affects the brain development and maturation. This injury is the result of the combination of a reduction in the cerebral oxygen (hypoxaemia) and/or a reduction of perfusion (ischaemia) and oxygenation to the brain. During the acute stage of brain damage, neuronal cell death occurs through necrosis, as a result of brain energy depletion, and even though metabolic energy levels recover in the brain after a few hours, a cascade of biochemical events is activated, resulting in neuronal apoptosis and, consequently, severe brain damage (Sanchez-Salcedo et al. 2019a). Therefore, the early failure of the energy supply represents an important factor in the survival of the new-born. Bearing in mind that the damage caused by asphyxia is progressive and dependent on the energy supply to the cells, one of the alternatives for therapeutic intervention is the administration of substances [such as: docosahexaenoic acid (DHA), thiamine pyrophosphate (PPT) and creatinine, among others] that act on the enzymatic pathways associated with energy production to reduce the magnitude of damage caused by perinatal asphyxia (Valenzuela-Peraza et al. 2014).

Therapy focused on metabolic energy levels with docosahexaenoic acid (DHA)

Docosahexaeonic acid (DHA) is an omega-3 fatty acid highly concentrated in the developing brain (Huun et al. 2018a). It is a constituent of the neuronal membranes in the grey matter, white matter and also in the glial cells. Fundamentally, it is crucial to maintain the ionic permeability of the membrane and the function of transmembrane receptors that support the synaptic transmission and cognitive abilities (Arteaga et al. 2015). Therefore, DHA has antioxidant properties and acts as a therapeutic agent. It has been shown to reduce inflammation,

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excitotoxicity, prevent brain volume loss in animal stroke models (Huun et al. 2018a) and improve the long-term neurological outcomes up to 5 weeks in neonatal rats that experienced hypoxic ischaemic injury during perinatal asphyxia (Arteaga et al. 2017a). Similarly, the administration of DHA, orally or subcutaneously, after ischaemic hypoxic injury has been shown to provide neuroprotection. For example, when DHA is administered in mothers' diets during pregnancy, it has been shown to provide neuroprotection in neonatal brain injury by inhibiting oxidative stress and apoptotic neuronal death (Suganuma et al. 2013). According to Arteaga et al. (2017b) DHA inhibits apoptotic neuronal death by minimising the production of ROS and by maintaining the mitochondrial integrity and function.

On the other hand, when DHA was administered intramuscularly alone or in combination with therapeutic hypothermia in porcine neonates presented with ischaemic hypoxic injury, a significant reduction in the oxidative stress was observed. This is because the administration of DHA causes a significant reduction in neuroprostanes F4 andisoprostane 8-iso-PGF2α (Huun et al. 2018b). Similarly, it causes a reduction in the cortical lactate/*N*-acetylaspartate (Lac/NAA) biomarker, which is positively correlated with abnormal neurological results (Huun et al. 2018a). Along these same lines, in another study carried out on seven-day-old neonatal rats with hypoxic ischaemic injury that underwent combined DHA (2.5 mg/kg) and therapeutic hypothermia [3 h, 30 °C], the combination of these treatments resulted in an improvement in the sensorimotor function and a reduction in the brain damage in neonates that sustained hypoxic ischaemic injury during perinatal asphyxia (Berman et al. 2013). DHA primarily decreases the brain damage by modifying the biophysical properties of the membrane and maintaining its integrity in functions between the presynaptic and postsynaptic areas, triggering the better stabilisation of the intracellular ion balance.

Although previous studies have shown that DHA therapies alone or in combination with therapeutic hypothermia have had beneficial effects in neonates with ischaemic hypoxic injuries, it is important to note that future studies will be needed to determine the optimal doses and routes of administration of DHA for post-ischaemic neuroprotection (Arteaga et al. 2015).

Therapy focused on metabolic energy levels with thiamine pyrophosphate (PPT)

During acute phases of hypoxic ischaemic encephalopathy (HIE), neuronal cell death by necrosis occurs because of a depletion of brain energy. Even at a few hours post-asphyxia, after the start of aerobic energy metabolism and replenishment of the glucose levels, the previously initiated apoptosis produces severe injury in the brain (Orozco-Gregorio et al. 2008; Sanchez-Salcedo et al. 2019a). Accordingly, one of the therapeutic agents studied for its possible neuroprotective effect is thiamine, a vitamin that exerts its metabolic action mainly through thiamine pyrophosphate (PPT), which is an indispensable cofactor in the activation of pyruvate dehydrogenase (PDH), alpha-keto glutarate dehydrogenase (α-CGDH), alpha-keto acid dehydrogenase and trancetolase; enzymes involved in energy generation processes through the Krebs cycle or the pentose pathway (Valenzuela-Peraza et al. 2014). Additionally, it has been observed that PPT has an important effect on the regulation of brain development during foetal and early postnatal life, therefore, it has an important effect on the psychomotor and sensory capacities of new-borns (Fattal-Valevski 2011). In a study by Valenzuela-Peraza et al. (2014) in neonatal rats (11-day-old) with experimentally induced hypoxia, it was found that, histologically, animals that received PPT one hour before suffering hypoxia exhibited less brain damage in the motor cortex, somatosensory cortex, and striated cortex in comparison with the control group. Likewise, it is important to note that, in this same study, it was shown that neonates who are administered PPT after hypoxia, exhibit tissue damage similar to animals in the control group. Therefore, these results suggest that once the cell death process has started, there is practically no possibility of reversing it. However, despite the above, it was observed that the administration of PPT either before or after the hypoxic event, normalises the pH, $PaCO_2$ and PaO_2 values. This shows that preserving the metabolic pathway that supplies the cell with energy could reduce the neuronal damage to some extent.

Additionally, more recent research carried out on rabbit foetuses by Jimenez-Bravo et al. (2016) showed that PPTis capable of modifying some biochemical parameters during ischemia *in utero* and during ensuing reperfusion. For example, the levels

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of foetal glucose were lower in foetuses without treatment in comparison to those foetuses that received PPT possibly as a result of preserving energy metabolism under hypoxic conditions. According to Valenzuela-Peraza et al. (2014), the neuroprotective effects of thiamine pyrophosphate (PPT), are due to the fact that PPT, being a highly energetic molecule, probably provides extra energy to the cell and produces the ATP that the cell needs to activate the ion channels and antioxidant systems of the brain, which are essential for the new-born to survive hypoxia and for the detoxification mechanisms to be activated. Therefore, the neuroprotective effects of PPT can be explained through: 1) The strengthening of the antioxidant system due to the capacity of PPT to capture free radicals, 2) The reduction in the excessive accumulation of excitotoxins involved in cell death such as glutamate and 3) The intracellular reduction in calcium accumulation (Valenzuela- Peraza et al. 2014; Sanchez-Salcedo et al. 2019a). Despite the fact that previous studies have shown that PPT has an effect only if it is administered before a hypoxic event, and although it is difficult to determine in advance whether or not an organism will be exposed to such an event in the neonatal stage, it would be interesting to assess the potential of PPT as a neuroprotective agent in high-risk situations such as, for example, in complicated delivery procedures and identification of foetal distress, among others (Valenzuela-Peraza et al. 2014).

Therapy focused on metabolic energy levels with creatine

Creatine (Cr) is a simple guanidine compound which is absorbed in the tissues via the creatine transporter and stored as creatine or phosphocreatine. Phosphocreatine is readily converted to creatine via creatine kinase in a reversible reaction which yields a high energy phosphate allowing the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) (Bain et al. 2013). Therefore, the Cr serves as an energetic buffer and through the Creatine/Phosphocreatine (PCr)/Creatine kinase (CK) system, and plays a critical role in the ATP metabolism of neurons. It also has the potential to suppress the generation of free oxygen radicals in the central nervous system that causes cell damage and inactivation of CK (Yar et al. 2015). In addition, in neurodegenerative animal models, creatine supplementation has been shown to reduce the oxidative stress, improve the brain functioning and attenuate the neuronal disintegration (Iqbal et al. 2015) and also improves the recovery of the cerebral blood flow after the cessation of a hypoxic episode (Bain et al. 2013). Hypoxic-ischaemic models of neonatal brain damage in rodents have provided support for the neuroprotective effects of creatine. Recent studies by LaRosa et al. (2016) in rats have shown that creatine supplementation (5% Cr) during pregnancy protects the muscles from asphyxia-induced damage at birth. A likely explanation of the protective effect of creatine treatment is that it slows down the rate of ATP depletion in tissues where it is most likely to occur because of a high dependence on ATP-related cellular processes. Creatine is also known to attenuate the accumulation of ROS and to produce vasodilatation, effects that might reduce the oxidative stress in muscles following the hypoxia and acidaemia caused by asphyxia at birth. In this vein, in another study carried out in female albino mice that underwent a supplementary diet of creatine at 1 and 3% after being weaned, it was found that supplementation with Cr significantly improves locomotory and exploratory behaviour after mice experienced hypoxic ischaemic injury during perinatal asphyxiation (Yar et al. 2015). This is probably due to the fact that the increase in the cellular levels of Cr causes ATP cell turnover to be maintained during the asphyxiation period and consequently prevents hypoxic ischaemic injury. Therefore, the results of these studies confirm that maternal dietary creatine supplementation during pregnancy could be a safe and readily available prophylactic treatment for the prevention of neonatal mortality and morbidity caused by perinatal asphyxia (LaRosa et al. 2016).

General discussion

Perinatal asphyxia is one of the main causes of mortality worldwide and it represents an important factor that can trigger physio-metabolic disorders and cause serious neurological damage not only in human foetuses and neonates, but also in different animal models. In recent years, the search for different pharmacological protocols to prevent and reverse physio-metabolic disorders and brain damage derived from perinatal asphyxia has been

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and continues to be the subject of intense research. Currently, within these pharmacological protocols that have been developed, psychoprophylactic therapies with caffeine, allopurinol, DHA, PPT, creatine, among other neuroprotective agents, have been shown to prevent the harmful effects of asphyxia in neonates. Thus, despite the fact that, currently, there are several investigations being carried out mainly on different animal models indicating that hypothermic therapies have become the standard treatment for human new-borns suffering from neonatal hypoxic-ischaemic encephalopathy after perinatal asphyxia (Thoresen et al. 2013), it is important to highlight that this therapy does not provide complete neuroprotection (Rodriguez-Fanjul et al. 2017) and is mainly administered as a treatment and not as a prophylactic therapy.

RECOMMENDATIONS

While there is an urgent need supported by all the scientific and clinical communities to find treatments and therapies that can prevent and/or lessen the effects of perinatal asphyxia, the goal has not yet been achieved, because this pathology has a multifactorial nature that makes it difficult to compare the different studies reported in the literature. For example, in animal model studies, the biofluid of choice, the sampling time, the cause of suffocation, the time of onset of asphyxia and its duration, and the analytical procedures are the most diversified. While in studies with human neonates, the main limitations are related to the low number of patients enrolled by a centre, the lack of an experimental group and the unequal choice of biofluids and tissues (Locci et al. 2020). Therefore, in future studies, it is recommended to deepen the validation of biomarkers and the knowledge of the pathways of action of these therapies and the development of clinical trials with a greater number of subjects where optimal doses and methodologies can be determined through the administration of these and other therapies.

FUTURE PROJECTIONS

Therefore, it is important to continue investigating the effects of new neuroprotective agents that serve as prophylactic therapies in different animal models to prevent the harmful effects of asphyxia. It is also important to highlight that emphasis is placed on research with animal models, since these have contributed to the knowledge and understanding of the pathophysiological mechanisms experienced by human neonates during perinatal asphyxia. Likewise, they have allowed the discovery of new pharmacological protocols (Yager 2004) and have been the basis for carrying out the practices that today are used as therapeutic strategies to treat and prevent the effects of asphyxia in human newborns. However, despite the above, animal models do not definitively encompass all aspects of human disease, since small animal models (e.g., mice, rats, and rabbits) are better at reproducing molecular events (Norflus and Gutekunst 2016), while large animal models (for example, piglets, sheep and primates) is important, because gyrencephalic brains of large animals (i.e., monkeys, sheep and pigs) permit the study of the selective cortical area and neuronal vulnerability, regional white matter injury, and connectivity. The amount of white matter in postnatal rodents is small, and the ability to perform biochemical assays on the white matter and cortical grey matter independently is not feasible. In contrast, infant monkeys and new-born pigs have white matter tractography similar to an infant human that differ only in quantity (Koehler et al. 2018).

Conclusion

Since asphyxia not only represents one of the main causes of perinatal mortality worldwide, but is also an important factor that can trigger physiological and metabolic imbalances and cause serious neurological consequences in neonates or foetuses. Research has been focused on developing effective therapies that can block clinical changes that are triggered (metabolic acidosis, hypercapnia, hypoxia, among others) and occur in the central nervous system during perinatal asphyxia. Currently, within these investigations, paediatricians and neuroscientists have observed neuroprotective effects and a significant decrease in mortality in several animal models and in human infants when respiratory stimulant therapies are administered (e.g., oxygen and caffeine), as well as hypothermic therapies alone or in combination with other putative agents (e.g., allopurinol, EPO, melatonin,

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N-acetylcysteine, noble gases) or therapies with energy supplements (e.g., DHA, PPT and Cr). For example, the administration of melatonin produces a powerful antioxidant and anti-inflammatory effect, acting as a free radical eliminator and regulating pro-inflammatory mediators; by inhibiting xanthine oxidase allopurinol can decrease oxidative stress. Erythropoietin has cell death and neurogenesis as its main therapeutic targets. Considering the whole scenario of the current therapies, the management of new-borns suffering from neonatal asphyxia could be based on the combination of one or some of these treatments, along with therapeutic hypothermia. Therefore, the current research with the aim of minimising the neurological sequelae derived from perinatal asphyxia seeks to develop new compounds that can be used synergistically with these therapies. On the other hand, recent research is beginning to include sex as a variable to be taken into account when treating physio-metabolic and neurological alterations that are triggered in perinatal asphyxia, as dimorphic sexual differences have been observed in both the mechanisms of damage (female experimental animals have shown greater memory deficits, while males are more susceptible to oxidative stress), as in the courses of action of the different treatments tested (i.e., caffeine or EPO). However, despite the promising progress made with these therapies, the development of preclinical studies is still required to deepen the knowledge of the pathways of action of these therapies and the development of clinical trials with a larger number of subjects in which the optimal doses and administration methodologies of these and other therapies can be determined.

Nevertheless, it is important to note that therapies with different neuroprotectors (such as xenon gas, 2-iminobiotin, topiramate or magnesium sulfate, autologous stem cells, among others) are currently being studied, which are considered as strategies to use in the intensive care of human infants with asphyxia. In addition, there are other therapies (such as flavonoids, cannabinoids, growth factors – BDNF: brain-derived neurotrophic factor or IGF-1: insulin-like growth factor type 1), which are currently further removed from clinical applications, since it is necessary to determine the extent of their neuroprotective capacity. Therefore, until a powerful strategy to improve the consequences of asphyxia is found, this topic will continue to be the subject of intense research in the future.

Conflict of interest

The authors declare no conflict of interest.

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