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REVIEW

Gender Differences Involved in the Pathophysiology of the Perinatal Hypoxic-Ischemic Damage

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Summary

Hypoxic-ischemic encephalopathy (HIE) is a neonatal condition that occurs as a consequence of perinatal asphyxia, which is caused by a number of factors, commonly *via* compression of the umbilical cord, placental abruption, severe meconium aspiration, congenital cardiac or pulmonary anomalies and birth trauma. Experimental studies have confirmed that male rat pups show a higher resistance to HIE treatment. Moreover, the long-term consequences of hypoxia in male are more severe in comparison to female rat pups. These sex differences can be attributed to the pathophysiology of hypoxia-ischemia, whereby studies are beginning to establish such gender-specific distinctions. The current and sole treatment for HIE is hypothermia, in which a reduction in temperature prevents long-term effects, such as cerebral palsy or seizures. However, in most cases hypothermia is not a sufficient treatment as indicated by a high mortality rate. In the present review, we discuss the gender differences within the pathophysiology of hypoxia-ischemia and delve into the role of gender in the incidence, progression and severity of the disease. Furthermore, this may result in the development of potential novel treatment approaches for targeting and preventing the long-term consequences of HIE.

Key words

Gender differences • Hypoxia • Hypoxic-ischemic encephalopathy • Immature brain

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Introduction to the concept of the perinatal hypoxia and ischemia

The term perinatal hypoxia-ischemia (HI) describes oxygen deprivation in immature, developing cerebral tissue. It is the 5th leading cause of mortality among children under five years and it is responsible for 23 % of neonatal deaths worldwide (Bryce *et al.* 2005, Lawn *et al.* 2005). More than two thirds of children who survive suffer from neurological impairments such as motor disabilities, seizures and developmental delays, where most of these signs are seen in the first few days of life (Millar *et al.* 2017). The newborn central nervous system develops robustly towards the adulthood and it is extremely sensitive to hypoxic conditions (Cuaycong *et al.* 2011). Mild hypoxic damage induced perinatally may remain unrecognized until adolescence (Inder and Volpe 2000). The pathophysiology of HI is accompanied by multiple processes, such as inflammation (Serdar *et al.* 2019), oxidative stress (Solevåg *et al.* 2019) and excitotoxicity (Doble 1999). These processes generally occur simultaneously and are one of the many reasons why the treatment of HI (or encephalopathy following these processes) is near impossible. One of the leading causes of HI is perinatal asphyxia – the reduction of blood flow to the immature brain (i.e. deprivation of oxygen). It initiates various processes in order to compensate for this oxygen deprivation, for example, redistribution of the blood flow to the vital organs (Giussani 2016). However, prolonged asphyxia (Fig. 1)

exhausts and decompensates such mechanisms (Rainaldi and Perlman 2019).

In addition, there are other predisposing factors that subject a neonate to a HI injury such as prolonged delivery, sepsis and shock. Subsequently, this can lead to cerebral palsy, cognitive deficits, respiratory distress and death (Allen and Brandon 2011). There is currently one established and main line therapy for HIE, which is the hypothermia. This involves cooling the neonatal brain to approximately 33 °C to slow the spread of cellular injury and minimize permanent damage (Shankaran *et al.* 2012, Pfister and Soll 2010). It is administered using either a cooling cap for head cooling or the whole-body-cooling for 72 h. This will slow the metabolic rate, allowing cells to recover and prevent further brain damage (Gluckman *et al.* 2005, Shankaran *et al.* 2005, Zhou *et al.* 2010). Despite the beneficial effect, one of the main issues is that hypothermia is limited by time; it should begin within 6 h following birth to minimize the spread of damage within the brain (Azzopardi *et al.* 2009, Diaz *et al.* 2017). Treatment delay from the diagnosis of HIE to the initiation of hypothermia may result in diminished therapeutic outcomes.

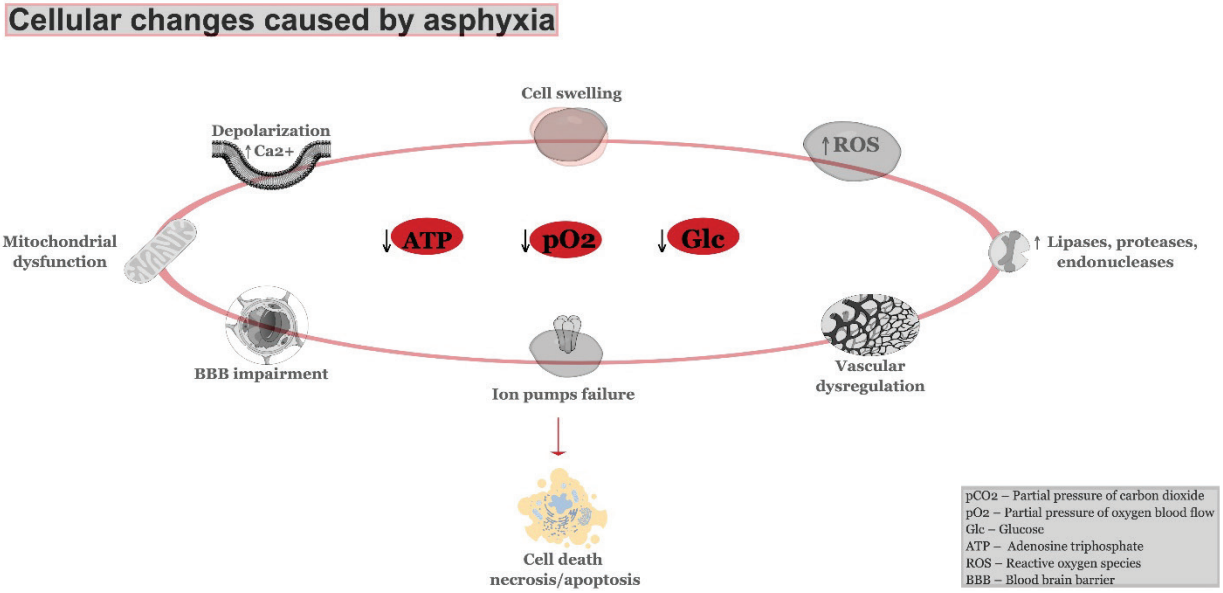

Fig. 1. Cellular changes caused by asphyxia.

Differences in metabolism between immature and adult brain

The immature brain of a neonate differs from an adult brain in response to HI injury. In addition to the role of gender, these attributes provide different modalities for potential treatment of HI. Firstly, the rate of metabolism is considerably lower in the immature neuronal tissue compared to an adult brain (Cremer 1982). Other metabolic substrates may be used as a metabolic fuel during the maturation of the brain; instead of glucose, ketone bodies and lactate should be used. These substrates cover more than half of the fuel that is required to satisfy the metabolic demands (Cremer 1982, Nehlig and Pereira de Vasconcelos 1993). Additionally, another significant difference between the adult and neonatal brain is the blood brain barrier morphology, where the immature brain shows a higher permeability, which further enhance brain damage in neonates (Millar *et al.* 2017). This is supported by experimental study of Muramatsu *et al.* (1997), reporting that rats at postnatal day (PND) 7 have higher permeability to immunoglobulin G compared to PND 14 rats (Muramatsu *et al.* 1997). The immature brain tissue could cope more effectively with the lack of metabolic substrate as compared to an adult brain tissue, but if a certain point of energy deprivation is reached, the immature brain tissue would suffer from excitotoxic damage (Puyal *et al.* 2013, Riljak *et al.* 2016). Destabilization of the cellular energy management is accompanied by the release of the excitatory amino acids and their failure of effective buffering in the extracellular space, which allows amino acids to reach their toxic concentrations (Doble 1999, Johnston 2005).

The role of the excitotoxicity, oxidative stress and inflammation in the development of HIE

Excitotoxicity, oxidative stress and inflammation are simultaneous processes during the setting of HI and each process can exacerbate neurological dysfunction resulting in encephalopathy, which is a non-specific response of the brain to injury (Vannucci *et al.* 1994, Riljak *et al.* 2016).

The production of reactive oxygen species (ROS) is a crucial outcome during ischemia (Folbergrová *et al.* 2016), which in turn, can trigger an inflammatory response. Reduction in blood flow, which last seconds to minutes, causes switching of the cell from aerobic to anaerobic metabolism, resulting in lactic acid formation, while fuel reserves decrease. This leads to the release of excitatory amino acids such as glutamate, whereby the intracellular concentration of calcium increases (Ferrer and Planas 2003). Glutamate binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), N-methyl-D-aspartate (NMDA) and kainate receptors resulting in tonic activation of these receptors. Consequently, more calcium can enter the cells, reaching a toxic level and an overexcitation of the cells. Excitotoxic amino acids (EAA) re-uptake mechanisms are failing and may be accompanied by cell depolarization and functional overload of the neurons. This can lead to rupturing and lysis of cells, which subsequently would initiate apoptotic cell death. Generation of free radicals, ROS, nitrogen species (NOS) and the release of calcium dependent enzymes such as caspases intensify the apoptotic activity of the cells (Velasco *et al.* 2017).

Excitotoxicity exacerbates inflammation, initially with the recruitment of inflammatory cells including phagocytes, monocytes and neutrophils. This is followed by the disruption of the blood brain barrier and development of brain oedema (Fellman and Raivio 1997). Brain oedema increases the intracranial pressure, leading to worsening of neurological dysfunction. This may result in hydrocephalus, brain stem herniation, respiratory distress and hemorrhages, the latter presenting a higher risk in males (Lang and McCullough 2008, Tioseco *et al.* 2006). Under physiological conditions, neurons and glial cells transform harmful superoxide into hydrogen peroxide, which forms water and oxygen i.e. benign molecules that can be utilized by the cells. However, a proportion of ROS remain unconverted and their excess may lead to the damage of neurons and glial cells resulting in apoptosis (Capani *et al.* 2001).

In addition, inflammatory cells can release other cytotoxic substances such as metalloproteinases, which serve as a source of ROS and cytokines such as IL-1, IL-6. This exacerbates the damages to the blood brain barrier and as a result, the disrupted blood brain barrier allows for other neurotoxic substances to enter leading to the formation of brain oedema (Kumar *et al.* 2008). Pro-inflammatory cytokines such as IL-1β can induce a rapid increase in excitability by activating its receptor, resulting in seizures (Vezzani *et al.* 2013, Youn *et al.* 2013), which is one of the long-term consequences of HIE.

Reduction in the partial pressure of oxygen (pO_2, Fig. 2) and an increase in the partial pressure of carbon dioxide affects significantly the ventilation. Only if the ventilation is restored, cerebral blood flow is matched with pO_2 and reperfusion prevents the exacerbation of oxidative stress and inflammation. If the ventilation is not

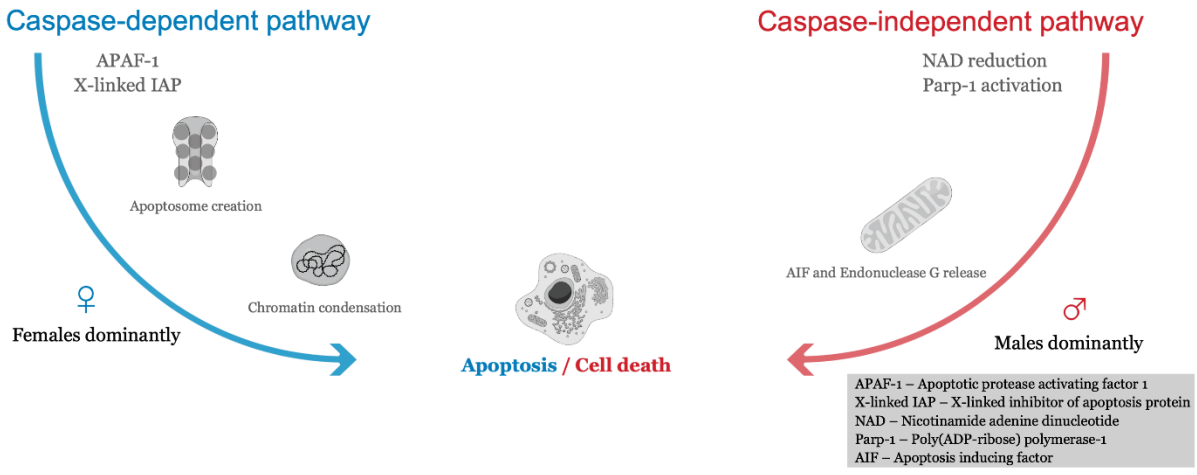

Fig. 2. Gender differences in apoptotic cascade triggered by HI.

restored adequately, the worsening of HI leads to the disruption of the blood brain barrier and consequently to excitotoxicity (Greer 2006, Kawabori and Yenari 2015, Kleman *et al.* 2010, Thorton *et al.* 2012).

Gender differences in severity of HIE

The unfavorable outcome of HIE in male infants compared to female infants have been established in ongoing research and demonstrated by radiological methods such as magnetic resonance imaging (MRI) and ultrasound (Gorelik *et al.* 2016, Jarvis *et al.* 2005, Johnston and Hagberg 2007, Zhu *et al.* 2006, Du *et al.* 2009, Hill and Fitch 2012, Hill *et al.* 2011a,b). The mortality rate of male infants is much higher than females, which suggests that one of the risk factors is gender. Stillbirths and respiratory distress syndrome are seen more in the male population (Zhao *et al.* 2017, Stephenson *et al.* 2000). Furthermore, male infants have a higher risk for blindness, deafness, developmental disorders including Autistic Spectrum Disorder and learning disabilities such as dyslexia, attention deficit disorder and cerebral palsy (Donders and Hoffman 2002, Hintz *et al.* 2007, Jarvis *et al.* 2005, Johnston and Hagberg 2007). Male infants are identified to be two times more likely to have prenatal anoxia, hemorrhages and an infection risk caused by cerebral birth trauma (Hill and Fitch 2012). The reason why males have a higher risk of cerebral birth trauma compared to females has not been investigated yet. However, MRI imaging has yielded positive results, showing premature male infants with a higher proportion of white matter injury compared to females, where the latter have their cerebral grey matter more vulnerable to hypoxia (Thompson *et al.* 2007). This was evident from rodent models of HIE where no sex difference was reported in the case of severe insult, however, in case of moderate insult females display less injury, which was attributed to differences in apoptosis (Zhu *et al.* 2006). MRI and ultrasound have shown that males following HI exhibited a higher brain volume loss, disrupted myelination and pronounced behavioral deficits, further supporting an exacerbated clinical picture in contrast to female neonates (Mayoral *et al.* 2009, Lan *et al.* 2011, Takeoka *et al.* 2002).

Contradictory to previous data, there is a study demonstrating lower mortality rate in males in comparison to females. A four-year study of newborns delivered in South-East Nigeria, demonstrated that females have higher mortality rate due to asphyxia. However, this study suffers from some limitations, as e.g. more male than female neonates were incorporated in this study (Ekwochi *et al.* 2017).

Cellular mechanisms coping with HI in both genders

On a cellular level, sexual dimorphism can be attributed to hormone-related actions, the apoptotic cascade differences and the so-called gene linked advantages. Higher levels of testosterone in males during the first year of life may enhance the process of neurotoxicity (Vannucci and Hurn 2009). The secretion of testosterone, which is the highest between gestational week 10-20, impacts brain development, providing a possible reason of a higher mortality rate in male neonates following cerebral injury. In addition, rat pups displayed a benefit effect from the depletion of testosterone following an injury, since the presence of testosterone increases glutamate toxicity (Yang *et al.* 2002, Hawk *et al.* 1998). This hypothesis is further supported by a study of Hill *et al.* (2011b) using the Rice-Vannucci rat model, where male and female rat pups received testosterone propionate (TP) from PND1 to PND5. There was a clear insufficiency in auditory processing of males, in both, treated with TP and without TP (Hill *et al.* 2011b). Female rat pups treated with TP had significantly worse auditory processing as compared to females without TP. Furthermore, there was a significant decrease in brain weight in males and TP treated females compared to their hypoxic and only vehicle counterparts, concluding that testosterone has detrimental consequences of early HIE (Hill *et al.* 2011b).

Estrogen has also demonstrated protective abilities against hypoxia and ischemia. Experimental studies have shown that female rodents have a lower incidence of stroke and less tissue damage than males (Yamori *et al.* 1977, Nuñez *et al.* 2007). This is further supported by a study from Fukuda *et al.* (2000), who have reported higher incidence of stroke in females following a reduction in estrogen levels induced by either ovariectomy or blockade of estrogen receptors, and also by age-related decline in estrogen production (Fukuda *et al.* 2000). This is, however, limited by the neonatal development, where there is a minimal level of circulating estrogen due to latent activation of ovaries in neonates. Thus, there must be additional protective factors leading to a lower severity of HIE in females (Johnston and Hagberg 2007).

Apoptotic cascades triggered by HI – gender effects

An encouraging line of investigation is directed at exploring gender differences in the mechanisms of cell death in response to brain injury. This has led to explore factors such as differences in apoptotic cascade, which consist of two pathways that may be preferentially activated depending on gender (Hill and Fitch 2012, Haast *et al.* 2012, Arambula *et al.* 2019, Hagberg *et al.* 2009). Programmed cell death is triggered by HI following deprivation of oxygen and glucose in neuronal cells (Northington *et al.* 2011). The range of damaging signals is initiated by a decrease in ATP and activation of neuronal nitric oxide synthase (nNOS). Along with release of excess EAA, there is prolonged activation and depolarization of NMDA and AMPA receptors, resulting in sodium and calcium influx, leading to cellular swelling and rapid cell death (Riljak *et al.* 2016, Zhu *et al.* 2006). There are two major pathways of apoptosis: the caspase-dependent pathway and caspase-independent pathway (Fig. 3). Following an increase in nNOS activation, the caspase-dependent pathway involves the signal of apoptotic protease activating factor 1 (APAF-1) and the formation of an apoptosome, which in turn, binds with caspases (3, 6, 7 and 9) resulting in chromatin condensation and DNA fragmentation. The caspase-independent pathway involves a reduction in nicotinamide adenine dinucleotide (NAD) and the activation of poly(ADP-ribose) polymerase-1 (Parp-1), leading to release of the apoptosis inducing factor (AIF) and endonuclease G from the mitochondrial compartments and ultimately, causing cell death (Zhu *et al.* 2006). Male or female gender favors either one of these pathways, but further research is required to determine why females rely more on the caspase-dependent pathway and males rely on the caspase-independent pathway following HIE (Lang and McCullough 2008, Renolleau *et al.* 2007, Zhu *et al.* 2006, Wang *et al.* 2004). On the contrary, previous studies showed that following HIE injury, there was a difference in the number of activated caspases. Males tend to have more caspases activated, which is subsequently associated with a wider apoptotic event and this may be attributed to a higher vulnerability in males, thus causing early brain damage (Netto *et al.* 2016, Liu *et al.* 2009, Hill and Fitch 2012). Inhibitors of apoptosis, in particular, Parp-1, has been extensively studied in relation to the caspase-independent pathway of apoptosis. Male rodents that were deficient in this enzyme, presented with a reduction in cerebral damage following stroke (Yuan *et al.* 2009). This is supported by another study, reporting lower inflammatory response in males, but not in females, following the inhibition of Parp-1 (Mabley *et al.* 2005). Thus, males preferentially undergo the caspase-independent pathway. Parp-1 and AIF were also found in higher concentration in the brains of male rodents at PND9 in comparison to female brains (Zhu *et al.* 2006). In female rodents, following a middle cerebral artery occlusion, the activation of a caspase-dependent apoptosis is preferred. Additionally, the inhibition of caspases-3 and the release of cytochrome-c resulted in a certain level of neuroprotection, observed in animal models (Zhu *et al.* 2006).

Moreover, the advantage of gene inheritance of endogenous apoptotic inhibitors such as X-linked

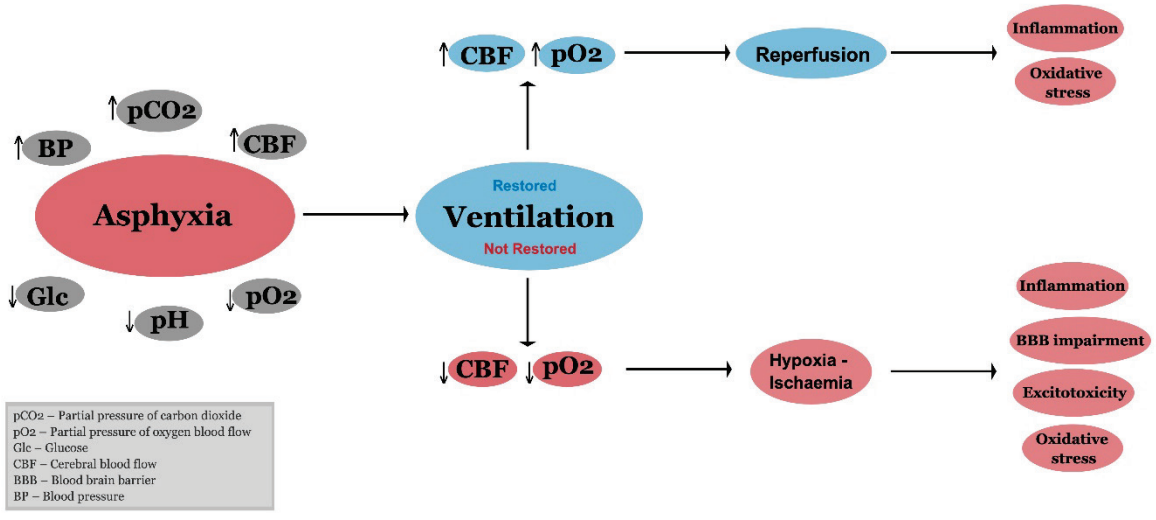

Fig. 3. Consequences of hypoxia and energy deprivation in HIE.

inhibitor of apoptosis protein (IAP) has been reported in females. Higher expression of X-linked IAPs in females may be beneficial during a HI insult, therefore cerebral damage is less extensive than in males (Deveraux and Reed 1999). The apoptotic cascade during development is highly regulated, thus, the need for anti-apoptotic signals promote the survival of neuronal cells. The IAPs are the most potent inhibitors that can effectively bind caspase-9 and prevent the down streaming of caspases, hindering cell death. The expression of X-linked IAPs has been observed in preclinical and clinical studies following HI injury (Askalan *et al.* 2011, Deveraux and Reed 1999). In comparison to males, females with a higher expression of X-linked IAPs preferentially activate apoptosis *via* caspases (Askalan *et al.* 2011).

Treatment – should it be gender targeted?

Despite the extensive research on sex differences in studies of neonatal HIE novel treatment approaches are not fully explored so far (Mayoral *et al.* 2009, Lan *et al.* 2011, Saraceno *et al.* 2010). Furthermore, a vast majority of studies (Hill and Fitch 2012, Yang *et al.* 2002) continue to utilize only male rodent models. However, this could be a possible source of bias reporting the worse clinical picture in males (Hill and Fitch 2012).

Hypothermia is the first line therapy of the HIE. Unfortunately, it is limited by sexual dimorphism as indicated by the high HIE mortality rate in male patients (Bona *et al.* 1998, Davidson *et al.* 2015, Hoehn *et al.* 2008).

Erythropoietin (EPO) is a new additive treatment currently used for premature infants as a treatment of anemia. EPO modulates NMDA excitotoxicity, reduces free radical toxicity, inflammation and improves the cognitive responses in primates and rats (Traudt *et al.* 2013, Davidson *et al.* 2015).

Melatonin is another perspective substance, which has been extensively tested within preclinical and clinical studies (Muller and Marks 2014). It has garnered some interest over the years due to being a free radical scavenger that can act in synergy with hypothermia to reduce oxygen deprivation in cerebral structures such as the hippocampus, where melatonin receptors are expressed to regulate myelination (Paprocka *et al.* 2019). Remarkably, the half-life of melatonin in preterm neonates is 15 h compared to adult brains which last up to 60 min. This significant difference in half-life makes melatonin a promising target for neuroprotection since it can possibly limit white matter injury, particularly in male rats (Park *et al.* 2014). It was proven beneficial if given synergistically with hypothermia as well (Park *et al.* 2014, Garg 2019).

Allopurinol is a xanthine oxidase inhibitor that can reduce the production of ROS, resulting in neuroprotection and improvement of HI (Rodriguez-Fanjul *et al.* 2017). In combination with hypothermia, this could be another potential treatment for HIE and its associated consequences. It has been shown (Fan *et al.* 2013, Nijboer *et al.* 2007), that following HI brain injury, females appear to benefit more from neuroprotective interventions (hypothermia, EPO and allopurinol). Nevertheless, studies also demonstrated that EPO and melatonin have a positive effect in males, providing a promising area of research into novel treatment for HI injury (Wen *et al.* 2006, Fang *et al.* 2013). There are many other substances that are now being considered to be as potential treatments in the therapy of HIE, such as topiramate, xenon and N-acetyl cysteine, however, more clinical and preclinical evidence are needed (Ozyener *et al.* 2012, Noh *et al.* 2006).

Conclusions

It is clear that gender is a crucial factor in hypoxia, its severity and the clinical outcome in neonates. Male neonates are at a higher risk of cerebral palsy as they showed more pronounced motor deficits than females. Moreover, the main line therapy with hypothermia may be affected by sex, since the outcome of HI is worse in males than females. Other treatments in combination with hypothermia may be promising at least in the rodent models and could be a potential treatment in clinical trials (e.g. EPO, melatonin and allopurinol). Thus, more therapies and novel gender-specific targets need to be investigated.

A vast majority of preclinical studies utilize male rodent models only, which consequently shows a worse clinical picture when compared to females. However, if there will be more female animal models used in experimental studies, it would bridge our understanding of sex-specific differences in hypoxia. Additionally, more research is required on the inherited role of IAPs and possibly on other apoptotic inhibitors in HI. Future studies would be focused on the relationship between steroid hormones and HI as there is a clear distinction in the concentration of sex hormones between males and females. Gene expression, along with the

apoptotic cascade represent a few of the many factors, which may play role in sex differences and are responsible for the disparities in severity of HIE. Future research might be focused on finding sex-specific neuroprotectants improving the outcome of neonatal HIE.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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References

ALLEN K, BRANDON D: Hypoxic ischemic encephalopathy: pathophysiology and experimental treatments. *Newborn Infant Nurs Rev* **11**: 125-133, 2011.

ARAMBULA SE, REINL EL, EL DEMERDASH N, MCCARTHY MM, ROBERTSON CL: Sex differences in paediatric traumatic brain injury. *Exp Neurol* **317**: 168-179, 2019.

ASKALAN R, WANG C, SHI H, ARMSTRONG E, YAGER JY: The effect of postischemic hypothermia on apoptotic cell death in the neonatal rat brain. *Dev Neurosci* **33**: 320-329, 2011.

AZZOPARDI D, STROHM B, EDWARDS D, DYET L, HENRY L, HALLIDAY H, EDMUND JUSZCZAK E, KAPELLOU O, LEVENE M, MARLOW N, PORTER E, THORESEN M, WHITELAW A: Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* **361**: 1349-1358, 2009.

BONA E, HAGBERG H, LØBERG EM, BÅGENHOLM R, THORESEN M: Protective effects of moderate hypothermia after neonatal hypoxia-ischemia: short- and long-term outcome. *Pediatr Res* **43**: 738-745, 1998.

BRYCE J, BOSHI-PINTO C, SHIBUYA K, BLACK RE: WHO estimates of the causes of death in children. *Lancet* **365**: 1147-1152, 2005.

CAPANI F, LOIDL CF, AGUIRRE F, PIEHL L, FACORRO G, HAGER A, DE PAOLI T, FARACH H, PECCI-SAAVEDRA J: Changes in reactive oxygen species (ROS) production in rat brain during global perinatal asphyxia: an ESR study. *Brain Res* **914**: 204-207, 2001.

CREMER JE: Substrate utilization and brain development. *J Cereb Blood Flow Metab* **2**: 394-407, 1982.

CUAYCONG M, ENGEL M, WEINSTEIN SL, SALMON E, PERLMAN JM, SUNDERAM S, VANNUCCI SJ: A novel approach to the study of hypoxia-ischemia-induced clinical and subclinical seizures in the neonatal rat. *Dev Neurosci* **33**: 241-250, 2011.

DAVIDSON JO, WASSINK G, VAN DEN HEUIJ LG, BENNET L, GUNN AJ: Therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy – where to from here? *Front Neurol* **6**: 1-10, 2015.

DEVERAUX QL, REED JC: IAP family proteins--suppressors of apoptosis. *Genes Dev* **13**: 239-252, 1999.

DIAZ J, ABIOLA S, KIM N, AVARITT O, FLOCK D, YU J, NORTHINGTON FJ, CHAVEZ-VALDEZ R: Therapeutic hypothermia provides variable protection against behavioral deficits after neonatal hypoxia-ischemia: a potential role for BDNF. *Dev Neurosci* **39**: 257-272, 2017.

DOBLE A: The role of excitotoxicity in neurodegenerative disease: implications for therapy. *Pharmacol Ther* **81**: 163-221, 1999.

DONDERS J, HOFFMAN NM: Gender differences in learning and memory after pediatric traumatic brain injury. *Neuropsychology* **16**: 491-499, 2002.

DU L, HICKEY RW, BAYIR H, WATKINS SC, TYURIN VA, GUO F, KOCHANEK PM, JENKINS LW, REN J, GIBSON G, CHU CT, KAGAN VE, CLARK RS: Starving neurons show sex difference in autophagy. *J Biol Chem* **284**: 2383-2396, 2009.

EKWOCHI U, ASINOBI NI, OSUORAH CD, NDU IK, IFEDIORA C, AMADI OF, IHEJI CC, ORJIOKE CJ, OKENWA WO, OKEKE BI: Incidence and predictors of mortality among newborns with perinatal asphyxia: a 4-year prospective study of newborns delivered in health care facilities in Enugu, South-East Nigeria. *Clin Med Insights Pediatr* **11**: 1-10, 2017.

FAN X, VAN BEL F, VAN DER KOOIJ MA, HEIJNEN CJ, GROENENDAAL F: Hypothermia and erythropoietin for neuroprotection after neonatal brain damage. *Pediatr Res* **73**: 18-23, 2013.

FANG AY, GONZALEZ FF, SHELDON RA, FERRIERO DM: Effects of combination therapy using hypothermia and erythropoietin in a rat model of neonatal hypoxia-ischemia. *Pediatr Res* **73**: 12-17, 2013.

FELLMAN V, RAIVIO KO: Reperfusion injury as the mechanism of brain damage after perinatal asphyxia. *Pediatr Res* **41**: 599-606, 1997.

FERRER I, PLANAS AM: Signaling of cell death and cell survival following focal cerebral ischemia: life and death struggle in the penumbra. *J Neuropathol Exp Neurol* **62**: 329-339, 2003.

FOLBERGROVÁ J, JEŠINA P, KUBOVÁ H, DRUGA R, OTÁHAL J: Status epilepticus in immature rats is associated with oxidative stress and mitochondrial dysfunction. *Front Cell Neurosci* **10**: 1-13, 2016.

FUKUDA K, YAO H, IBAYASHI S, NAKAHARA T, UCHIMURA H, FUJISHIMA M, HALL ED: Ovariectomy exacerbates and estrogen replacement attenuates photothrombotic focal ischemic brain injury in rats. *Stroke* **31**: 155-160, 2000.

GARG BD: Melatonin as a neuroprotective agent in hypoxic ischemic encephalopathy: is it beneficial: *EC Paediatrics* **8**: 53-60, 2019.

GIUSSANI DA: The fetal brain sparing response to hypoxia: physiological mechanisms. *J Physiol* **594**: 1215-1230, 2016.

GLUCKMAN PD, WYATT JS, AZZOPARDI D, BALLARD R, EDWARDS AD, FERRIERO DM, POLIN RA, ROBERTSON CM, THORESEN M, WHITELAW A, GUNN AJ: Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* **365**: 663-670, 2005.

GORELIK N, FAINGOLD R, DANEMAN A, EPELMAN M: Intraventricular hemorrhage in term neonates with hypoxic-ischemic encephalopathy: a comparison study between neonates treated with and without hypothermia. *Quant Imaging Med Surg* **6**: 504-509, 2016.

GREER D: Mechanisms of injury in hypoxic-ischemic encephalopathy: implications to therapy. *Semin Neurol* **26**: 373-379, 2006.

HAAST R, GUSTAFSON D, KILIAAN A: Sex differences in stroke. *J Cereb Blood Flow Metab* **32**: 2100-2107, 2012.

HAGBERG H, MALLARD C, ROUSSET C, WANG X: Apoptotic Mechanisms in the Immature Brain: Involvement of Mitochondria. *J Child Neurol* **24**: 1141-1146, 2009.

HAWK T, ZHANG YQ, RAJAKUMAR G, DAY AL, SIMPKINS JW: Testosterone increases and estradiol decreases middle cerebral artery occlusion lesion size in male rats. *Brain Res* **796**: 296-298, 1998.

HILL CA, ALEXANDER ML, MCCULLOUGH LD, FITCH RH: Inhibition of X-linked inhibitor of apoptosis with embelin differentially affects male versus female behavioral outcome following neonatal hypoxia-ischemia in rats. *Dev Neurosci* **33**: 494-504, 2011a.

HILL CA, FITCH RH: Sex differences in mechanisms and outcome of neonatal hypoxia-ischemia in rodent models: implications for sex-specific neuroprotection in clinical neonatal practice. *Neurol Res Int* **2012**: 1-9, 2012.

HILL CA, THRELKELD SW, FITCH RH: Early testosterone modulated sex differences in behavioral outcome following neonatal hypoxia ischemia in rats. *Int J Dev Neurosci* **29**: 381-388, 2011b.

HINTZ S, KENDRICK D, VOHR B, POOLE W, HIGGINS R: Gender differences in neurodevelopmental outcomes among extremely preterm, extremely-low-birthweight infants. *Acta Paediatr* **95**: 1239-1248, 2007.

HOEHN T, HANSMANN G, BÜHRER C, SIMBRUNER G, GUNN AJ, YAGER J, LEVENE M, HAMRICK SE, SHANKARAN S, THORESEN M: Therapeutic hypothermia in neonates. Review of current clinical data, ILCOR recommendations and suggestions for implementation in neonatal intensive care units. *Resuscitation* **78**: 7-12, 2008.

INDER TE, VOLPE JJ: Mechanisms of perinatal brain injury. *Semin Neonatol* **5**: 3-16, 2000.

JARVIS S, GLINIANAIA SV, ARNAUD C, FAUCONNIER J, JOHNSON A, MCMANUS V, TOPP M, UVEBRANT P, CANS C, KRÄGELOH-MANN I: Case gender and severity in cerebral palsy varies with intrauterine growth. *Arch Dis Child* **90**: 474-479, 2005.

JOHNSTON MV: Excitotoxicity in perinatal brain injury. *Brain Pathol* **15**: 234-240, 2005.

JOHNSTON MV, HAGBERG H: Sex and the pathogenesis of cerebral palsy. *Dev Med Child Neurol* **49**: 74-78, 2007.

KAWABORI M, YENARI M: Inflammatory responses in brain ischemia. *Curr Med Chem* **22**: 1258-1277, 2015.

KLEMAN N, DUN D, CENGIZ P: Mechanisms underlying neonatal hypoxia ischemia. *Open Drug Discov J* **2**: 129-137, 2010.

KUMAR A, MITTAL R, KHANNA HD, BASU S: Free radical injury and blood-brain barrier permeability in hypoxic-ischemic encephalopathy. *Pediatrics* **122**: 722-727, 2008.

LAN A, LIAO X, MO L, YANG C, YANG Z, WANG X, HU F, CHEN P, FENG J, ZHENG D, XIAO L: Hydrogen sulfide protects against chemical hypoxia-induced injury by inhibiting ROS-activated ERK1/2 and p38MAPK signaling pathways in PC12 cells. *PLoS One* **6**: 1-14, 2011.

LANG JT, MCCULLOUGH LD: Pathways to ischemic neuronal cell death: are sex differences relevant? *J Transl Med* **6**: 1-10, 2008.

LAWN J, SHIBUYA K, STEIN C: No cry at birth: global estimates of intrapartum stillbirths and intrapartum-related neonatal deaths. *Bull World Health Organ* **83**: 409-417, 2005.

LIU F, LI Z, LI J, SIEGEL C, YUAN R, MCCULLOUGH L: Sex differences in caspase activation after stroke. *Stroke* **40**: 1842-1848, 2009.

MABLEY JG, HORVÁTH EM, MURTHY KG, ZSENGELLÉR Z, VASLIN A, BENKO R, KOLLAI M, SZABÓ C: Gender differences in the endotoxin-induced inflammatory and vascular responses: potential role of poly(ADP-ribose) polymerase activation. *J Pharmacol Exp Ther* **315**: 812-820, 2005.

MAYORAL SR, OMAR G, PENN A: Sex differences in a hypoxia model of preterm brain damage. *Pediatr Res* **66**: 248-253, 2009.

MILLAR L, SHI L, HOERDER-SUABEDISSEN A, MOLNAR Z: Neonatal hypoxia ischaemia: mechanisms, models and therapeutic challenges. *Front Cell Neurosci* **11**: 78, 2017.

MULLER AJ, MARKS JD: Hypoxic ischemic brain injury: Potential therapeutic interventions for the future. *Neoreviews* **15**: 177-186, 2014.

MURAMATSU K, FUKUDA A, TOGARI H: Vulnerability to cerebral hypoxic-ischemic insult in neonatal but not in adult rats is in parallel with disruption of the blood-brain barrier. *Stroke* **28**: 2281-2289, 1997.

NEHLIG A, PEREIRA DE VASCONCELOS A: Glucose and ketone body utilization by the brain of neonatal rats. *Prog Neurobiol* **40**: 163-221, 1993.

NETTO C, SANCHES E, ODORCYK F, DURAN-CARABALI L, WEIS S: Sex-dependent consequences of neonatal brain hypoxia-ischemia in the rat. *J Neurosci res* **95**: 409-421, 2016.

NIJBOER CH, GROENENDAAL F, KAVELAARS A, HAGBERG HH, VAN BEL F, HEIJNEN C: Gender-specific neuroprotection by 2-iminobiotin after hypoxia-ischemia in the neonatal rat via a nitric oxide independent pathway. *J Cereb Blood Flow Metab* **27**: 282-292, 2007.

NOH MR, KIM SK, SUN W, PARK SK, CHOI HC, LIM JH, KIM IH, KIM HJ, KIM H, EUN BL: Neuroprotective effect of topiramate on hypoxic ischemic brain injury in neonatal rats. *Exp. Neurol* **201**: 470-478, 2006.

NORTHINGTON FJ, CHAVEZ-VALDEZ R, MARTIN LJ: Neuronal cell death in neonatal hypoxia-ischemia. *Ann Neurol* **69**: 743-758, 2011.

NUÑEZ J, YANG Z, JIANG Y, GRANDYS T, MARK I, LEVISON SW: 17beta-estradiol protects the neonatal brain from hypoxia-ischemia. *Exp Neurol* **208**: 269-276, 2007.

OZYENER, F, CETINKAYA, M, ALKAN T, GOREN B, KAFA IM, KURT MA, KOKSAL N: Neuroprotective effects of melatonin administered alone or in combination with topiramate in neonatal hypoxic-ischemic rat model. *Restor Neurol Neurosci* **30**: 435-444, 2012.

PAPROCKA J, KIJONKA M, RZEPKA B, SOKÓŁ M: Melatonin in hypoxic-ischemic brain injury in term and preterm babies. *Int J Endocrinol* **2019**: 1-11, 2019.

PARK JH, KIM CS, LEE SL, LEE SR: The effect of combination treatment of melatonin and hypothermia on hypoxic-ischemic brain injury in neonatal rats. *Neonatal Med* **21**: 129-137, 2014.

PFISTER RH, SOLL RF: Hypothermia for the treatment of infants with hypoxic-ischemic encephalopathy. *J Perinatol* **160**: 82-87, 2010.

PUYAL J, GINET V, CLARKE PG: Multiple interacting cell death mechanisms in the mediation of excitotoxicity and ischemic brain damage: a challenge for neuroprotection. *Prog Neurobiol* **105**: 24-48, 2013.

RAINALDI MA, PERLMAN JM: Pathophysiology of birth asphyxia: *Clin Perinatol* **43**: 409-422, 2016.

RENOLLEAU S, FAU S, GOYENVALLE C, JOLY LM, CHAUVIER D, JACOTOT E, MARIANI J, CHARRIAUT-MARLANGUE C: Specific caspase inhibitor Q-VD-OPh prevents neonatal stroke in P7 rat: a role for gender. *J Neurochem* **100**: 1062-1071, 2007.

RILJAK V, KRAF J, DARYANANI A, JIRUŠKA P, OTÁHAL J: Pathophysiology of perinatal hypoxic-ischemic encephalopathy – biomarkers, animal models and treatment perspectives. *Physiol Res* **65**: 1-13, 2016.

RODRIGUEZ-FANJUL J, FERNANDEZ-FEIJO C, LOPEZ-ABAD M, RAMOS M, CABALLE R, HORRILLO A, CAMPRUB M: Neuroprotection with hypothermia and allopurinol in an animal model of hypoxic- ischemic injury: Is it a gender question? *PLoS One* **12**: 1-16, 2017.

SARACENO GE, BERTOLINO ML, GALEANO P, ROMERO JI, GARCIA-SEGURA LM, CAPANI F: Estradiol therapy in adulthood reverses glial and neuronal alterations caused by perinatal asphyxia. *Exp Neurol* **223**: 615-622, 2010.

SERDAR M, KEMPE K, RIZAZAD M, HERZ J, BENDIX I, FELDERHOFF-MÜSER U, SABIR H: Early pro-inflammatory microglia activation after inflammation-sensitized hypoxic-ischemic brain injury in neonatal rats. *Front Cell Neurosci* **13**: 237, 2019.

SHANKARAN S, LAPTOOK AR, EHRENKRANZ R, TYSON J, McDONALD S, DONOVAN, FANAROFF A, POOLE W, WRIGHT L, HIGGINS R. FINER N, CARLO W: Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* **353**: 1574-1584. 2005.

SHANKARAN S, LAPTOOK AR, TYSON JE, EHRENKRANZ RA, BANN CM, DAS A, HIGGINS RD, BARA R, PAPPAS A, McDONALD SA, GOLDBERG RN, WALSH MC: Evolution of encephalopathy during whole body hypothermia for neonatal hypoxic-ischemic encephalopathy. *J Pediatr* **160**: 567-572, 2012.

SOLEVÅG AL, SCHMÖLZER GM, CHEUNG PY: Novel interventions to reduce oxidative-stress related brain injury in neonatal asphyxia. *Free Radic Biol Med* **142**: 113-122, 2019.

STEPHENSON R, MOHAN RM, DUFFIN J, JARSKY TM: Circadian rhythms in the chemoreflex control of breathing. *Am J Physiol Regul Integr Comp Physiol* **278**: R282-R286, 2000.

TAKEOKA M, SOMAN TB, YOSHII A: Diffusion-weighted images in neonatal cerebral hypoxic-ischemic injury. *Pediatr Neurol* **26**: 274-281, 2002.

THOMPSON DK, WARFIELD SK, CARLIN JB, PAVLOVIC M, WANG HX, BEAR M, KEAN MJ, DOYLE LW, EGAN GF, INDER TE: Perinatal risk factors altering regional brain structure in the preterm infant. *Brain* **130**: 667-677, 2007.

THORTON C, ROUSETT C, KICHEV A, MIYAKUNI Y, VONTELL R, BABURAMANI A, FLEISS B, GRESSENS P, HAGBERG H: Molecular mechanisms of neonatal brain injury. *Neurol Res Int* **2012**: 1-16, 2012.

TIOSECO J, ESSERS A, PATEL K, EL-MOHANDES A: Male sex and intraventricular haemorrhage. *Paediatr Crit Care Med* **7**: 40-44, 2006.

TRAUDT CM, McPHERSON RJ, BAUER LA, RICHARDS TL, BURBACHER TM, McADAMS RM, JUUL SE: Concurrent erythropoietin and hypothermia treatment improve outcomes in a term nonhuman primate model of perinatal asphyxia. *Dev Neurosci* **35**: 491-503, 2013.

VANNUCCI SJ, HURN PD: Gender differences in pediatric stroke: is elevated testosterone a risk factor for boys? *Ann Neurol* **66**: 713-714, 2009.

VANNUCCI SJ, MAHER F, KOEHLER E, SIMPSON IA: Altered expression of GLUT-1 and GLUT-3 glucose transporters in neurohypophysis of water-deprived or diabetic rats. *Am J Physiol* **267**: 605-611, 1994.

VELASCO M, ROJAS-QUINTERO J, CHÁVEZ-CASTILLO M, ROJAS M, BAUTISTA J, MARTÍNEZ MS, SALAZAR J, MENDOTA R, BERMÚDEZ V: Excitotoxicity: an organized crime at the cellular level. *J Neurol Neurosci* **8**: 1-10, 2017.

VEZZANI A, ARONICA E, MAZARATI A, PITTMAN QJ: Epilepsy and brain inflammation. *Exp Neurol* **244**: 11-21, 2013.

WANG X, ZHU C, WANG X, HAGBERG H, KORHONEN L, SANDBERG M, LINDHOLM D, BLOMGREN K: X-linked inhibitor of apoptosis (XIAP) protein protects against caspase activation and tissue loss after neonatal hypoxia-ischemia. *Neurobiol Dis* **16**: 179-189, 2004.

WEN T, ROGIDO M, PENG H, GENETTA T, MOORE J, SOLA A: Gender differences in long-term beneficial effects of erythropoietin given after neonatal stroke in postnatal day-7 rats. *Neuroscience* **13**: 803-811, 2006.

YAMORI Y, HORIE R, AKIGUCHI I, NARA Y, OHTAKA M, FUKASE M: Pathogenic mechanisms and prevention of stroke in stroke-prone spontaneously hypertensive rats. *Prog Brain Res* **47**: 219-234, 1977.

YANG SH, PEREZ E, CUTRIGHT J, LIU R, HE Z, DAY AL, SIMPKINS JW: Testosterone increases neurotoxicity of glutamate in vitro and ischemia-reperfusion injury in an animal model. *J Appl Physiol* **92**: 195-201, 2002.

YUAN M, SIEGEL C, ZENG Z, LI J, LIU F, MCCULLOUGH LD: Sex differences in the response to activation of the poly (ADP-ribose) polymerase pathway after experimental stroke. *Exp Neurol* **217**: 210-218, 2009.

YOUN Y, SUNG IK, LEE IG: The role of cytokines in seizures: interleukin (IL)-1β, IL-1Ra, IL-8, and IL-10. *Korean J Pediatr* **56**: 271-274, 2013.

ZHAO F, YANG J, CUI R: Effect of hypoxic injury in mood disorder. *Neural Plast* **2017**: 1-10, 2017.

ZHOU WH, CHENG GQ, SHAO XM, LIU XZ, SHAN RB, ZHUANG DY, ZHOU CL, DU LZ, CAO Y, YANG Q, WANG LS: Selective head cooling with mild systemic hypothermia after neonatal hypoxic-ischemic encephalopathy: a multicenter randomized controlled trial in China. *J Pediatr* **157**: 367-372, 2010.

ZHU C, XU F, WANG X, SHIBATA M, UCHIYAMA Y, BLOMGREN K, HAGBERG H: Different apoptotic mechanisms are activated in male and female brains after neonatal hypoxia-ischaemia: *J Neurochem* **96**: 1016-1027, 2006.