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Review Article

Stem Cell Research on Potential Treatment Method for Neonatal Brain Disorder Hypoxic Ischemic Encephalopathy

Elizabeth F Donohue and Vincent S Gallicchio*

Department of Biological Sciences, Clemson University, USA

***Corresponding Author:** Vincent Gallicchio, Department of Biological Sciences, College of Science, Clemson University, USA.

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Abstract

Despite the groundbreaking scientific breakthroughs occurring today, Hypoxic Ischemic Encephalopathy (HIE) is still one of the most significant causes of fatalities of neonates worldwide. HIE is a type of brain injury that results in neonates losing oxygen and blood flow to the brain. There is currently only one form of treatment for HIE, induced hypothermia. This type of treatment works by delaying the depolarization of anoxic cells so that the affected cell will not go into apoptosis as quickly as it would without treatment. The issue with this treatment is that it is not reliable and only works on neonates with mild to moderate cases of HIE, with little impact on severe cases. Current research suggests mesenchymal stem cells are potential new treatment for HIE due to their ability to be able to differentiate into different types of cells. This review discusses the current research being conducted on stem cells as a potential addition to the already existing treatment method of induced hypothermia, as well as the possible complications and restrictions of using stem cells as a potential treatment method.

Keywords: Stem Cell Research; Therapeutic Hypothermia; Neonatal; Hypoxic Ischemic Encephalopathy; UCB Derived Stem Cells

Introduction of HIE and Current Treatment Methods

There have been many recent advances in neonatal intensive care medicine and treatment options [1]; however, Hypoxic Ischemic Encephalopathy (HIE), one of the most prominent neonatal disorders, is still without an effective treatment/cure [1]. HIE causes 23% of deaths in neonates worldwide, and is the fifth leading cause of death in children under the age of five [2]. HIE, or intrapartum asphyxia, is defined as the deprivation of oxygen and blood flow to the brain for a period of time dependent on the cause of the injury and the endogenous response from the body in neonates [3]. HIE affects an estimated twenty out of every one thousand live births around the world, making it the most common type of brain damage that occurs in neonates [2]. Additionally, this type of brain damage results in 10-60% chance of death in neonates after birth [2].

HIE is caused by a variety of effects that allow carbon monoxide to enter the brain and restrict the flow of oxygen to the brain and from it [4]. There are many different ways that a neonate can experience this condition, which include the neonate undergoing cardiac arrest at some point during gestation, suffering from respiratory arrest, and/or experiencing near-drowning [4]. When oxygen supply to the brain is compromised, it causes apoptosis of the neurons, oligodendrocytes, and adipocytes located in the affected part of the brain [5]. This injury can occur in different degrees of variation ranging from mild to severe. The severity depends on the mechanisms that caused HIE as well as the protocols that are taken to mitigate the damage done [6]. Some of the effects that occur as a result of the severity of HIE are: cerebral palsy, mental retardation, learning disabilities, and even epilepsy [6]. After the onset of HIE, an inflammatory response occurs in the brain which is the primary cause of brain damage in the neonate [3]. The inflammatory response is due to a leukocyte influx that develops due to the diminished oxygen and blood supply to the brain [3]. This inflammatory response in the body is mediated by cytokines, a large group of signaling proteins, peptides, or glycoproteins

in the body that are secreted by cells of the immune system. These signaling molecules regulate immunity, inflammation, and hematopoiesis in the body to allow for minimal inflammation to occur in order to prevent more damage being done [3].

Recovery from HIE depends on certain factors, such as the affected areas in the brain as well as the initial cause of the brain damage in the neonate [4]. A full recovery from HIE is extremely rare and some studies have found that there is a 50% chance of mortality of the neonates that survive with HIE [1], as well as a 25% chance that the neonates that do survive will permanently display cerebral palsy or another type of severe long term brain damage [1]. According to Ahn and his colleagues, there are three stages of HIE: primary/acute phase, latent phase, and a secondary phase [1]. The primary/acute phase represents the stage in which some of the neuronal cells undergo apoptosis and the other cells undergo recovery. The second phase, the latent phase, starts the process of the recovery of cellular respiration. The third and final stage of HIE, the secondary phase, occurs only when there has been moderate to severe brain damage to the neonate as a result of HIE [1]. If this stage does occur in a neonate, it starts several hours after onset (6-15 hours later). The secondary stage is defined by the failure of the mitochondrial oxidative activity of a cell, which is the main reason as to why cell death occurs [1].

HIE is one the leading cause of death in neonates [7]; however, as of 2016 there only has been one treatment option that is not very reliable, therapeutic hypothermia [7]. Therapeutic hypothermia is only effective on mild to moderate cases of HIE, so it is necessary to search for a new more efficient treatment option. Current research proposes that mesenchymal stem cells from a variety of locations in the body may be a viable treatment option. Mesenchymal stem cells can easily differentiate into many various types of somatic cells, including osteoblasts and adipocytes [8]. The most promising stem cell treatment is umbilical cord derived mesenchymal cells [7]. Another possible treatment method is the combination of therapeutic hypothermia and stem cells as a viable treatment option [9]. Finding a new treatment option that allows for a timely and effective solution is important because after a certain amount of time, HIE becomes completely irreversible and results in permanent severe brain damage throughout an individual's lifetime. Due to the intensity and the prominence of HIE, researchers need to further investigate new treatment options for this type of neonatal brain damage.

Current treatment method: therapeutic hypothermia

The only current mode of treatment for neonates with Hypoxic Ischemic Encephalopathy is therapeutic hypothermia [7]. This form of treatment, which is considered a neuroprotective technique used to treat neonates with perinatal asphyxia and HIE, is only effective in mild to moderate cases and is considered a neuroprotective technique used to treat neonates with perinatal asphyxia and HIE [1]. Perinatal asphyxia is defined as a medical condition in which the neonate's oxygen supply is compromised for a period of time dependent on the cause of injury and the endogenous response, which causes abnormal breathing patterns [10]. Therapeutic hypothermia works by cooling the neonate's head to around 34.5°C and the body to around 33.5°C [3]. The treatment is effective because it reduces the amount of excitatory amino acids, which then causes anoxic cells to delay depolarization [1].

Ahn and his colleagues speculated that there is a time window that allows for the greatest effect in disrupting the process of HIE [1]. Therapeutic hypothermia now is induced within six hours after birth and must be maintained for seventy-two hours. Recent research recommends that this type of treatment should be implemented directly following the latency stage and before the secondary phase for the best chance of neuronal recovery in a neonate with HIE.

However, as previously stated, therapeutic hypothermia is not reliable and is only effective in the milder cases of HIE. Additionally, there are also some possible side effects that are associated with implementing therapeutic hypothermia too early, when the neonate is still in the womb, or when the temperature is not monitored closely enough and kept steady for the seventy-two hours [3]. If therapeutic hypothermia is induced while the neonate is still in the womb, there is a chance that it will cause detrimental effects in the neonates, such as seizures, cerebral palsy, or an even worse chance at survival once born [3]. If the temperature is not monitored closely enough after inducing hypothermia, then the neonate can develop permanent side effects that affect their blood, such as hypertension, blood clotting, and thrombocytopenia [3]. Some of these effects are not due directly to hypothermia but rather the side effects of multiorgan dysfunction. In addition to early onset side effects, Ahn and his colleagues are exploring the longitudinal effects on individuals who have been treated with therapeutic hypothermia as a neonate, as they have suffered from

detrimental health effects that may have been caused by this treatment [1]. Therapeutic hypothermia is not effective enough to treat HIE alone, especially in the more severe cases. There are too many potential life-altering side effects to have this treatment as the primary option for this brain disorder that is one of the biggest contributors to neonatal death each year [2].

Clinical trial on therapeutic hypothermia

In April of 2008, Laptook and colleagues started their eight year research project regarding the effect of therapeutic hypothermia initiated after six hours in neonates with HIE. The clinical trials were conducted on 168 neonates that were thirty weeks or later in gestation with moderate to severe HIE [11]. Therapeutic hypothermia was induced between six to twenty four hours after birth in the neonates. The neonates were placed into an experimental group and a control group. The experimental group was cooled to 33.5°C for 96 hours and then rewarmed. The control group was maintained at 37.0°C for the duration of the experiment [11]. The results of this study demonstrated that between the experimental group and the control group, the neonates in the experimental group showed a 76% reduction in the probability of death or permanent damage after birth, and a 64% chance of at least a 2% decrease in death or damage in eighteen to twenty-two months. The overall conclusion of this study suggested that therapeutic hypothermia contains benefit, but it is unclear of how effective it is on its own.

Free radicals and treatment methodology

In order to find a new treatment method for HIE, researchers must understand the pathophysiology of the brain [12]. This knowledge will permit researchers to be able to detect when a neonate is at risk for HIE or another brain disorder due to the lack of oxygen or blood to the brain. Scientists need to find a treatment option that reduces the amount of free radicals that are produced as an effect of therapeutic hypothermia, as well as contain anti-inflammatory and anti-apoptotic factors [13]. Free radicals are uncharged molecules that contain an unpaired valence electron [12]. They are usually highly reactive and have a short lifespan because they find stability after searching for other stable molecules to steal a valence electron from, which causes a chain reaction [12]. They are highly reactive and can react with important cellular components, like DNA or the cell membrane [12]. This process will cause the cell to start functioning poorly. In extreme cases, the cell can even undergo apoptosis and die [14]. Free radicals are formed due to a variety of components: exercise, inflammation, ischemia, and radiation. When there is a balance of free radicals and antioxidants in the body, then the body will function normally [12]. Antioxidants target free radicals in order to ensure the balance and to keep from free radicals from taking over too many cells in the body [12]. They are constantly forming in the body as a result of the enzymatic and non-enzymatic processes that the cell undergoes. The body undergoes a natural cycle of cell death and cell birth, and free radicals help with creating new cells and killing off old ones [14].

Stem cells

Recent research suggests that stem cells combined with therapeutic hypothermia might be a new and viable treatment option for Hypoxic Ischemic Encephalopathy [1]. Studies completed by Park and colleagues [7], Wang and colleagues, and Ahn and colleagues have investigated the potential use of three types of stem cells: umbilical cord, neural, and mesenchymal respectively [9]. All three of these stem cell sources provide advantages, such as: neural stem cells are capable of regeneration of brain cells and mesenchymal stem cells are able to differentiate into a variety of cells throughout the body, and umbilical cord stem cells are present at birth in the umbilical cord and the placenta and are available to be stored in liquid nitrogen to be able to save these cells for potential future clinical use within the family [15]. However, these three stem cells also display disadvantages as well, such as: neural stem cells have a limited ability to regenerate and repair cells in the Central Nervous System, mesenchymal stem cells are not capable of total body regeneration, and umbilical cord stem cells engraft at a slower rate into the individual after transplant [16]. Out of the three stem cells mentioned the most promising is umbilical cord stem cells [7]. They are easily obtainable from the umbilical cord at birth and contain high levels of pluripotent stem cells, meaning they have the ability to grow into the primary germ layers: ectoderm, endoderm, and mesoderm. The most glaring issue with this stem cell is that unless the umbilical cord and placenta are saved at birth, these stem cells are harder to come by for adult clinical use [16].

The second potential stem cell as a viable treatment option, neural stem cells, have the ability to generate glial cells and other neuronal cells of the nervous system. This would allow for the generation of cells that are lacking in the brain due to the onset of

HIE. The biggest issue with neural stem cells is that they are not naturally present in the developing brain [9]. Furthermore, they do not survive in the brain well alone. The final stem cell treatment option that research suggests would be effective in treating HIE is the use of mesenchymal stem cells (MSCs) [1]. An positive aspect of these stem cells is that they are less susceptible to infection as a result of the introduction of new antigens being introduced due to their lack of the major histocompatibility complex (MHCII) antigens [1]. This is a positive trait because it helps mesenchymal stem cells from avoiding allogeneic rejection. When stem cells are transplanted into an individual from a host individual, a possible complication is Graft vs. Host Disease (GVHD). GVHD occurs when the lymphocytes of the donor attack the immune system of the host [15]. Due to the lack of the MHCII complex in MHCs, they are less susceptible to GVHD. Additionally, MSCs allow for greater allogeneic therapy, meaning they are able to produce a large batch of stem cells from unrelated donor tissues. Recent studies have shown that MSCs that are derived from all birth related tissues are the most promising treatment method to be tested in future clinical research [1].

There has only been one completed human clinical trial researching stem cells as a treatment option for HIE to date [5]; however, researchers have conducted animal studies with neural stem cell transplantation in mice [9], and umbilical cord blood transplantation in rats [7]. The research demonstrated that the optimal time for treatment with stem cells would be on the seventh day after the onset of HIE [1]. The optimal route for transplantation of the stem cells into the patients is still being determined [1]. Several modes of transplantation: intraventricular, intrathecal, and intravenous have all been used in the past with cell-based treatments [1]. Intraventricular and intrathecal administrations are typically more invasive because they require the direct injection of stem cells into the brain. Most drug injections are performed intravenously to reduce the potential risks that accompany direct injections due to this injection method allowing flow through the bloodstream [1]. Research proposes that even though intraventricular and intrathecal administration of stem cells is more invasive, it is the better option due to neonates containing an open anterior fontanelle. These methods allow for the injection to be administered directly into the damaged area(s) of the brain [1].

While the use of stem cells are a promising new treatment method for HIE, ethical concerns are currently deterring the progression of further research, especially research with the use of embryonic stem cells [1]. The public has raised moral concerns regarding the status of the embryo when the embryonic stem cells are gathered. In order to be able to combat some of the moral stigma that surrounds the use of embryonic stem cells, researchers have created oversight committees [17]. Another concern with use of embryonic stem cells is their ability to develop teratomas [17]. A third potential concern of the usage of stem cells by including healthy individuals in clinical trials, as this can compromise the value of the data derived [17]. These concerns have made finding funding for stem cell research difficult; therefore, slowing the progression of further research into determining whether or not stem cells would be an effective treatment method for HIE in neonates [17]. However, there have been a few beneficial animal trials performed by researchers concerning stem cell use as a potential treatment for HIE. Park and his colleagues conducted a study that examined the use of umbilical cord blood (UCB) derived mesenchymal cells in rats [7]. Additionally, Wang and colleagues also performed a study that examined the use of neural stem cells in mice [9]. Both of these experiments highlighted some potential issues with stem cell use, and additionally discussed some possible breakthroughs for a new treatment method. Both studies showed that the treatment method of combined treatment with therapeutic hypothermia and the respective stem cell used was highly effective [7,9].

Potential treatment method: umbilical cord blood (UCB) derived mesenchymal cells

Park and his colleagues conducted their research on the use of derived mesenchymal cells from umbilical cord blood in rats [7]. These scientists hypothesized that the best treatment method for HIE would be to combine the already existing treatment of therapeutic hypothermia with human umbilical cord blood (UCB) derived MSCs [7]. UCB derived MSCs were chosen due to their availability and their ability to avoid triggering cell-mediated immune responses in the body. Additionally, these stem cells have been proven to decrease the severity of HIE in rats that encompases over 50% of the ipsilateral hemisphere [7]. This type of stem cells has been used in the past as a treatment method for patients with cancer or other genetic disorders [5].

Park and his colleagues conducted their experiment by blocking off the rat's blood supply in the carotid artery in order to induce HIE in over 50% of the ipsilateral hemisphere [7]. After the onset of HIE, they subjected rats with an oxygen level of 8% for two hours. Animals were divided into four different groups: HIE combined with normothermia and MSC (HNM), HIE combined with normothermia control (HNC), HIE combined with hypothermia control (HHC), and normoxia normothermia control (NNC) [7]. This study consisted of six experiments that tested various common brain disabilities in patients with HIE, including: apoptosis, lack of inflammatory cytokines in the brain, impaired sensorimotor functioning, brain inflammation, and more. All six of the experiments that were conducted resulted in the HNM group showing the most improvement, showing that the combined treatment method worked the best at combating these common brain disabilities [7].

From this study, scientists concluded that the animals that were subjected to the combined treatment method of therapeutic hypothermia and UCB derived MSCs showed the most improvement in all of the experiments conducted. Additionally, they concluded that the ultimate determinant of the success of therapeutic hypothermia is the severity of HIE and not the timing of the treatment method [7].

Phase 1 clinical trial

As of 1 Mar 2017, there has only been one recorded phase 1 trial using autologous UCB cells in neonates. The study was conducted by Cotten and colleagues conducted a study on 23 neonates receiving concurrent treatment with therapeutic hypothermia [5]. The 23 neonates received intravenous infusion of 4 doses of 1-5 x 10^7 cells/dose of autologous UCB cells. There were no significant adverse reactions or infections that occurred after the transfusion of the UCB cells [5]. The results of the study concluded that after one year, the neonates who received the combined treatment of UCB with therapeutic hypothermia showed a 74% survival rate, while the neonates that received treatment with therapeutic hypothermia alone showed a 41% survival rate [5]. Phase 1 clinical trials conducted in neonates have shown to be safe, feasible, and potentially effective in treating neonates with Hypoxic Ischemic Encephalopathy [18].

Potential treatment method: neural stem cells

Another study investigating the potential of the use of stem cells as an option for neonatal HIE treatment concerned neural stem cells [11]. Wang and colleagues performed their experiment on mice, and they hypothesized the combination of therapeutic hypothermia and neural stem cells would provide a more effective treatment method than either method alone [9]. Neural stem cells have the ability to generate nervous system cells in the CNS that have been damaged by HIE, showing improvements in learning and ability in the patient [9].

The mice in the study were placed into three experimental groups based on the treatment method they would receive: neuronal stem cell (NSC), hypothermia, and a combined treatment method of NSC with hypothermia [9]. Of all the important experiments conducted on the mice, one of the most important was inducing HIE into the brain of the mouse and then twenty-four hours later dissecting the terminal deoxynucleotidyl transferase nick end labeling (TUNEL)-positive cell to detect DNA fragments that were formed due to apoptosis as a result of HIE in the brain [19]. The group that showed the lowest number of DNA fragments was the combined treatment method of NSCs and hypothermia. This result is due to NSCs having the ability to differentiate into different neural cells and travel to the damaged areas in the brain [9]. Therapeutic hypothermia prevents blood-brain barrier contaminations, reduces metabolic rates of cells, and decreases apoptosis occurrences in cells [9]. Additionally, it stops the NSCs from dying so that they can settle into the areas of the brain that require them [9]. The combination method allows for the generation of required cells in the brain as well as the decrease in apoptosis in cells, decreasing the damaged areas of the brain that have been affected by HIE.

Conclusion

The only current treatment method for HIE is therapeutic hypothermia; however it is not reliable and is only effective in mild to moderate cases of HIE. A newer and more effective treatment method is needed to treat neonates with HIE and other neonatal brain disorders, as the damage is a large contributor to the neonatal deaths that occur each year. Researchers are exploring the potential use of stem cells, especially mesenchymal stem cells, as an additional treatment method to be used in combination with therapeutic hypothermia in order to provide the neonate with the greatest chance at survival. Stem cells are a viable and realistic

option due to their availability and ability to differentiate into different cell types. The most heavily researched stem cell option is mesenchymal stem cells that are derived from birth associated tissues.

In the laboratory studies conducted on rats treated with umbilical cord stem cells and mice treated with neural stem cells, there were multiple experiments on the potential long term brain effects of HIE that can be reduced by the use of stem cells and therapeutic hypothermia as a combined treatment method, and the benefits of each [7,9]. All of the current research concluded that a combined treatment method provides the neonate with the greatest chance at survival; however, additional animal trials need to be conducted before more human trials can be considered. Future studies need to be done in order to determine the best optimal dosage, transplantation method, optimal timing of the injection of the stem cells, and the proper safety procedures that need to be followed for the injection of neural, umbilical cord, or mesenchymal stem cells under hypothermic conditions, as well as the best location to derive stem cells from to be able to use in neonates for treatment. The combination of stem cells and therapeutic hypothermia is a promising method of treatment, and with further animal trials and research, it can be considered for additional human clinical trials someday in the near future. There remains to be only one completed phase 1 clinical trial conducted on neonates that occurred in 2013 [5]. The results of the study remain promising for future research and experimentation on the use of stem cells as a potential treatment method in combination with therapeutic hypothermia in neonates with Hypoxic Ischemic Encephalopathy [5]. Future human clinical trials should reflect the animal trials conducted with neural and umbilical cord stem cells with the combined treatment method being tested. Additional human trials cannot start until there have been further experimentation in a laboratory setting and the ethical concerns of stem cell usage in neonates is resolved.

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