Mesenteric Neural Stem Cell for Chronic Spinal Cord Injury: A Literature Review

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Abstract

Spinal cord injury (SCI) is a common and potentially life-threatening condition with no established treatment to treat the primary injury. Mesenteric neural stem cell (NSC) therapy is a promising stem cell therapy to treat primary SCI in the chronic phase. We aimed to review the literature narratively to describe current evidence regarding mesenteric NSC in SCI. Primary SCI refers to tissue damage that occurs at the time of trauma that leads to the death of neuronal cells. In chronic SCI, the ability of neuronal regeneration is compromised by the development of gliotic scar. NSC is a stem cell therapy that targeted SCI in the chronic phase. Enteric NSC is one of the sources of NSC, and autologous gut harvesting in the appendix using endoscopic surgery provides a more straightforward and low-risk procedure. Intramedullary transplantation of stem cell with ultrasound guiding is administration technique which offers long-term regeneration. Mesenteric NSC is a promising stem cell therapy to treat chronic SCI with low risk and easier procedure to isolate cells compared to other NSC sources.

Introduction

Spinal cord injury (SCI) is a potentially life-threatening condition and requires a high long-term treatment cost. It commonly occurs in young adult and traffic accidents; falls from height, violence, and sport-associated injury are the common cause of injury [1]. The pathophysiology of SCI is very complicated, consisting of primary, secondary, and tertiary injuries. Primary injury is the injury caused directly by the trauma itself, while the secondary and tertiary injury is the effects of physical and biochemical responses against the primary injury [2]. At present, the established treatment for SCI is focusing on preventing secondary and tertiary injury. There is no established treatment to treat the primary injury. Therefore, the development of more effective treatments, or better yet, cures for SCI, is of paramount importance.

Stem cell therapy is a promising therapy to replace death cells due to primary injury of SCI. The use of several stem cell sources for SCI therapy has been attempted with varying degrees of success, including non-neural lines. A variety of stem cell sources have shown great potential toward achieving partial, if not complete, functional recovery following SCI.

Enteric neural stem cells (ENSCs), isolated from the adult gut, are a promising alternative stem cell source. These are the resident stem cells of the ENS and have been isolated from patients up to 80 years [3]. They are derived from neural crest cells (NCCs), a transient population of cells that give rise to numerous cell types throughout the body, including the ENS of the gastrointestinal tract [4]. Transplantation of these cells into the gastrointestinal tract shows that they can differentiate into neurons and glial cells [5]. Recently, ENSCs cultured from postnatal human tissue have been shown to retain similar migratory, proliferative, and differentiation capabilities to embryonic neural crest-derived cells, demonstrating their potential benefits toward tissue regeneration in SCI following transplantation [6]. In this article, we describe the pathophysiology of SCI and its relation to the concept of

stem cell therapy in SCI. Following that, we discussed the ENSCs as potential NSCs to treat SCI.

SCI and Pathophysiology

The main issue regarding the pathophysiology of SCI is tissue damage [7]. Following SCI, tissue injury is divided into two main phases, primary and secondary injury [8]. Primary injury refers to tissue damage that occurs at the time of trauma that leads to the death of neuronal cells. A secondary injury is a progressive event that happens after primary injury and lasts for a few weeks, months, or years. Several temporal phases of SCI can be divided into five categories depending on time relative to the primary injury. Chronic SCI is classified as an injury that is occurred within 6 months after the primary injury that has the potential either functional or structural plasticity of the spared spinal cord [2], [9].

Many theories explain secondary injury in SCI, such as apoptosis, ischemia, excitotoxicity, inflammation, mitochondrial dysfunction, and oxidative cell damage similar to traumatic brain injury [2]. Endogenous repair processes aim to isolate the lesion to expand and clear necrotic tissue. The repair process involves inflammation, infiltration of leucocyte, glial scar formation (astrogliosis), and programmed cell death [10]. Uncontrolled activity of these secondary mediators leads to the exacerbation of the injury, creating an inhibitory chemical and physical milieu that prevents endogenous efforts of remyelination, plasticity, regeneration, and repair. The mature central nervous system (CNS) is incapable of neurogenesis. However, the study found a localization in which neurogenesis was active around the subgranular and subependymal layers of the hippocampus.

One of the mechanisms in chronic SCI was the development of gliotic scar and cerebrospinal fluid (CSF) accumulation inside the spinal cord caused "fluid-filled cavities," also called syringomyelia (Figure 1) [11]. Traumatic syringomyelia develops for about 4.5% of patients with SCI [12]. Many theories tried to explain the pathogenesis of syringomyelia, such as intramedullary pulse pressure proposed by Greitz [12]. Nowadays, the theory explaining syringomyelia resulted from abnormal CSF hydrodynamics within the subarachnoid space of the spinal caused by spinal trauma leads to the ischemic lesion and spinal cystic degeneration, thus forming a larger cavity. Other theories explain that atrophy of the spinal cord will force the central canal to expand, force spinal cord parenchyma outward, and disturb CSF hydrodynamic circulation. CSF cannot circulate within the central canal and would prefer to enter spinal cord parenchyma and form "fluid-filled cavity." Diffuse obstruction of the subarachnoid space may also induce syringomyelia [13].
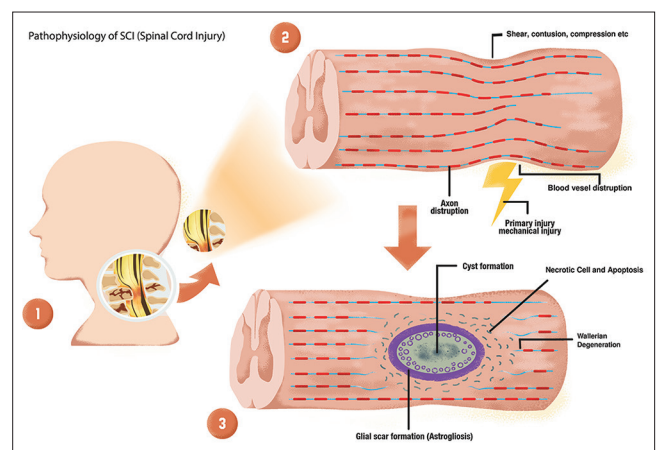

Figure 1: Schematic pathophysiology of spinal cord injury (SCI) and development of gliotic scar, which compromises the axonal regeneration in SCI

The presence of gliotic scar and traumatic syringomyelia inhibits the growth of axons through the lesional tissue. This problem is challenging because the gliotic scar is one of the endogenous repair mechanisms of SCI while it also inhibits the growth of transplanted stem cells. Efforts should be made to degrade the gliotic scar to improve the growth of axons through lesional tissue.

Concept of Stem Cell Therapy in SCI

The strategy of stem cell transplantation in SCI is executed based on the phase of the SCI itself. As we know, there are immediate, acute, subacute, and chronic phases of SCI. The goals of stem cell treatment are different according to the phase of SCI. In the immediate phase, the goal is reducing cell death due to trauma, while in the acute phase, the goal is increasing neuroprotection. In the subacute phase, the goal is tissue modification, and in the chronic phase, the target of stem cell treatment is cell repair through neuroplasticity [10].

At present, several sources of cells have been studied for the treatment of SCI, including mesenchymal stem cells (MSCs), peripheral myelinated cells (PMCs), and NSCs. The sources of MSCs are bone marrow stromal cells, umbilical cord blood, and umbilical cord matrix cells. The PMCs are originating from ensheathing olfactory cells and Schwann cells. The source of NSCs comes from adult and fetal tissue, embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs) (Figure 2) [10].

Transplantation of MSCs in SCI results in immunomodulation and trophic support in injured SCI, which caused an increase in endogenous neuron regeneration [14]. However, MSCs did not produce new neurons to replace the loss of neurons due to injury.
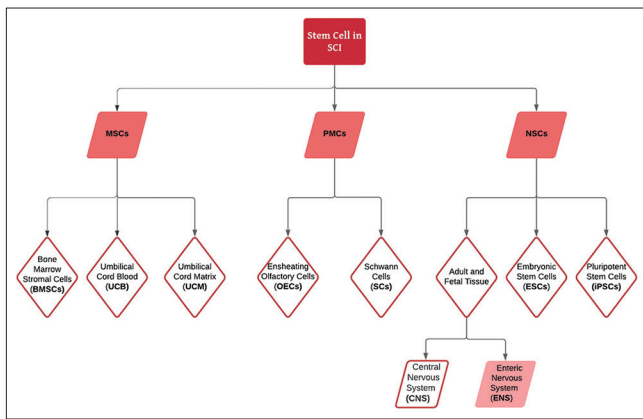
Figure 2: Schematic diagram of stem cell therapy sources for spinal cord injury treatment

Transplantation of PMCs plays a role in remyelination and tissue scaffolding. It is reported that transplantation of PMCs has resulted in improved motor and sensory function [15]. However, the source of these cells is from an isolated area of the brain and must be removed surgically, which presents a high additional risk to the patient [10].

The transplantation of NSCs provides regeneration through cell replacement and neuroplasticity (Figure 3). As known, multipotent NSCs are located in the periventricular regions of the CNS, especially the lining of the ventricles. In particular, the NSCs are from the ependymal layer in the subventricular and subgranular zones of the brain and the subependymal layer of the central canal in the spinal cord [10]. Another source of NSCs is the ESCs. The ESCs line is very versatile and easily differentiates into many germ cells of the mesoderm, endoderm, and neuroectoderm. However, the versatility and ability to differentiate into many cell lines provide another problem. The main concern of ESCs transplantation is the risk of tumor formation and immune rejection. One of the undifferentiated features of ESCs is their ability to form teratomas and has been shown to activate the innate immune response.

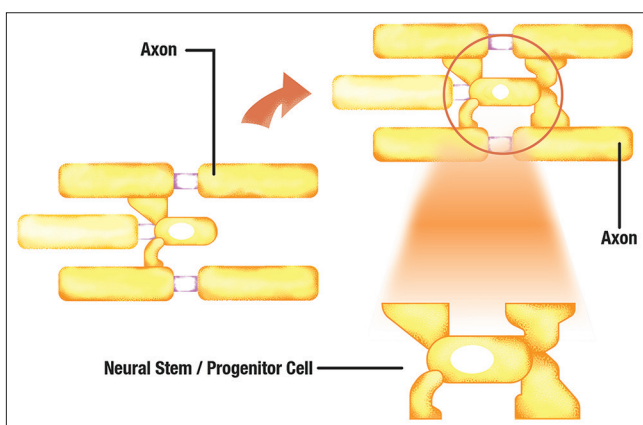Advance in technology gives rise to advancement in iPSCs technology and have opened up new potential therapeutic approaches. The iPSCs line is created by returning somatic cells to a pluripotent state through upregulation of four transcription factors, which are OCT4 and SOX2, with KLF4 and C-MYC. However, the reprogramming method is still inefficient and requiring more than 6 months for manufacture and differentiation to produce a patient-specific product [10].

ENSC

In adults, NSCs have been restricted to specific areas in the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus of the hippocampus [16], [17]. Harvesting and isolation of CNS-derived NSCs from adults are only possible through highly invasive surgery or under surgical indications. This kind of procedure is a high-risk procedure, which precludes their use for clinical translation [10].

At present, the NSCs can be harvested and isolated from the peripheral nervous system (PNS), which reduces the risk of cell harvesting and isolation. The enteric nervous system (ENS) is the part of the PNS that controls critical aspects of bowel function, including peristalsis, regulation of blood flow, and secretion of water and electrolytes. The adult ENS consists of neurons and glial cells arranged as ganglia in two concentric rings, the myenteric and submucosal plexus, which lie between the layers of smooth muscle [18].

ENSCs are persistent ENS stem cells isolated from the intestine of an adult [3]. ENSCs originate from NCCs that migrate from the vagal and sacral nerve tubes that colonize the developing intestine [18], [19]. Progressive differentiation from the stem cells into neurons and glial cells and further organization into ganglia occur in the intestine environment [20]. ENSCs have been shown to maintain similar migration, proliferation, and differentiation capabilities to cells derived from embryonic nerve crest. These capabilities are demonstrating their potential benefits to neural tissue regeneration [6].

As we mentioned above, the goal of stem cell treatment in chronic SCI is regeneration through neuroplasticity. Plasticity is the ability of the nervous system to rewire its connection or adapt its functions to the actual microenvironmental situation [21]. ENS expressed growth-associated protein 43, which is a protein that is correlated to neuronal growth and regeneration. This protein is highly expressed in the myenteric and submucous ganglia, thus giving evidence for a lifelong capability of the ENS to adapt to new challenges [22].

The characteristic of ENS and CNS must be similar to permit ENSCs to be successfully transplanted

Figure 3: Schematic picture describing role of neural stem cell in cell regeneration during chronic spinal cord injury

in the injured spinal cord. Although functionally and morphologically different, both CNS and ENS share many similar essential characteristics. The ENS and CNS are connected physically and in constant communication through the gut-brain axis. Higher brain centers exert control to the intestine through either vagal pathways, sympathetic pathways, or pelvic pathways. Communication between ENS and CNS is bidirectional, with axons of ENS projecting to the spinal cord [23]. The close interaction and information transfer between CNS and ENS strongly suggest that transplanted ENSCs would be able to integrate with endogenous spinal cord neurons to restore function.

Another factor that can assess the similarity between CNS and ENS is the expressed protein by progenitor cells in CNS and ENS. Neurotrophin receptor p75 is expressed in stem cells found at the subventricular zone and the progenitor cell retrieved from the submucosal and myenteric plexus of the intestine [24]. The other marker expressed in CNS and ENS is SRY-box-2 (Sox2). Sox2 is an essential protein in maintaining pluripotency by suppressing transcription factors that guide NSC to differentiate. Sox2 is also taking part in the neurogenesis of progenitor cells [25]. A cell that expresses a high level of Sox2 has been shown to maintain self-regeneration capacity and produce a neurosphere [26].

The other important marker that is commonly used to identify the NSC population is nestin. Nestin is a filament protein that is important in sustaining the life of NSC. When removed, the NSC without nestin will increase the apoptotic process and decrease self-renewal potency. Both ENS and CNS progenitor cells highly expressed nestin [27], [28], [29].

Another study also reported that the differentiation of NSCs into neuron or glial cells resulted in identical expression of protein from the neuron, oligodendrocyte, and astrocyte in both CNS and ENS. The identical expressed proteins in the neuron are Tuj1, PGP9.5, nNOS, and neurofilament, while GFAP and S100 are the protein identically expressed by glial cells in both CNS and ENS [30], [31], [32].

Besides expressing similar progenitor protein, neurotransmitters utilized by cells in ENS must be similar to CNS. In the literature, the ENS also utilizes almost all the neurotransmitters similar to the CNS [33]. However, the level of several neurotransmitters is different between the spinal cord and intestine. The neurotransmitters are acetylcholine, serotonin, gamma-aminobutyric acid, nitric oxide, nNOS, and glutamate [33], [34]. Levels of nNOS, serotonin, glutamine, and acetylcholine were reportedly similarly high between the spinal cord and intestine. Expression of neurotransmitter GABA was significantly higher in the spinal cord compared to the intestine [34]. Neurons of the myenteric plexus produce serotonin but in more modest quantities [35].

The bidirectional communication between ENS and CNS, followed by similar protein expression and neurotransmitters, suggests that both nervous systems have similar characteristics. The transplantation of ENSCs into the injured spinal cord is possible because of this similarity. It is reported that ENSCs readily differentiated toward a neuronal phenotype both *in vitro* and *in vivo* following transplantation. The neuronal phenotype showed a high expression of Tuj1, which is a neuronal marker. The transplanted ENSCs were reported expressed Tuj1+, which suggest a potential source to replace neuron in SCI [34].

The transplanted ENSCs have the ability to extend axons alongside spinal cord-derived cell axons to function as bridges. This is supported by the extension of axons toward one another leads to the formation of synaptic junctions. The GFP+ staining within the injury zone overlapped with endogenous Tuj1+/GFP- staining, showing a synergistic relationship between the spinal cord and the transplanted cells, which encourage survival through the formation of "bridges." Transplanted ENSCs appeared to cross the glial scar demonstrating differentiation to appropriate lineage. They did not seem to cause overtly increased activation or proliferation of astrocytes or macrophages/microglia [34].

Autologous Gut Harvesting

Autologous gut harvesting must be performed to obtain ENSCs. Theoretically, all the gastrointestinal systems can be harvested to obtain ENSCs. However, we should consider the risk and benefit regarding the location of autologous gut harvesting. The appendix is a good site that can be used for harvesting ENSCs [36]. Appendix does not have any essential function to human physiology, and it is located in an easily accessible area. Appendectomy is not harmful, even in a diseased patient, and with minimally invasive surgical techniques, the patient's risk is decreased further. Adult ENS can be repeatedly accessed through routine endoscopy to generate an autologous cell source for transplant, thus avoiding the harvesting/ethical issues that hinder other NSC sources.

Cells derived from dissociated gut tissues can be cultivated and expanded. This process will be giving rise to neurons and glial cells [37]. These cells not only have proliferation and differentiation potential but are also capable of migrating [38]. The techniques developed to isolate ENS progenitors involve selection on the basis of cell surface marker expression, culture with factors favoring progenitor cell growth, or selection on the basis of proliferative potential [37], [39].

Co-cultivation of ENSs with mesenteric vascular cells (MVCs) facilitates neuronal regeneration

and tube formation [40]. The generated neurospheres and dissociated cells showed characteristics of NSCs shown by expression of nestin, Nanog, Sox2, and Oct4. The differentiating and mature neurons are also shown by the expression of ß-Tubulin III and tyrosine hydroxylase [41].

As described by Hagl _et al_. [41], after separation of submucous and muscular layer, the tissue is digested enzymatically with collagenase II solution (Worthington) for 4 h. Isolation of pure myenteric (MP) and submucous plexus was performed, and the collected ganglia were mildly dissociated with Accutase for 20 min and plated in 25 cm^2 culture flasks at standardized densities (16,106 cells/flask) using a standard neuronal medium. After 6 days _in vitro_, free-floating enteric neurospheres (EnNSs) abounded while differentiating neurons and glia cells at the bottom could easily be discriminated. The supernatant with EnNSs and differentiation neuronal and glial cells was cultivated further. The EnNSs cultures were maintained up to 40 days at 37°C in a humified atmosphere.

Route of Stem Cell Transplantation

The basic concept that we should know regarding the various route of stem cell transplantation is the ability of stem cells to migrate. It is already known that NSC can migrate to the site of SCI and differentiate into neurons and glial cells, replacing damaged cells to treat SCI [38], [42]. ENSCs are derived from enteric NCCs and known to be a highly migratory population [43], [44], [45]. One of the superiorities of ENSC-derived cells is using the injury as a localization cue. Because of this behavior, the ENSC localization to the injured site in SCI could be expected following transplantation [34].

Regarding transplantation, there are several pathways currently being used by the researcher to transplant the stem cells into the SCI model. In general, the route of stem cell transplantation can be divided into systemic and local administration. The systemic routes of stem cell transplantation are intravenous, transarterial, transnasal, and intraperitoneal. The local route of stem cell transplantation is intrathecal and intramedullary injections [45].

Intravenous administration of stem cells is not an invasive procedure and does not damage the spinal cord tissue. However, because of systemic administration, this route needs many stem cells to be administered at once to fulfill adequate migration of stem cells to the injured site [46]. Besides the intravenous route, intra-arterial administration can be used to transplant the stem cell also. The stem cells can also migrate to the injured site following this route. However, even though it is not invasive, the intravascular route also has a dangerous disadvantage, such as easy to cause blood vessel embolism.

Intranasal administration can also be used to administer stem cells to the injured spinal cord. It was reported that this route could also cause stem cells to migrate to the injured spinal cord. The administered stem cells reduce the intramedullary cavity and the recovery of hind limb motor function. However, the therapeutic effect following this procedure is not as significant as that of intrathecal administration [47]. Intraperitoneal route can also be used to transplant stem cells. A study by Ramalho _et al_. compared the intraperitoneal and intravenous stem cell transplantation and found that the two approaches had similar therapeutic effects on the treatment of SCI [48].

Local administration of stem cells to the injured part is better than systemic administration because of direct transplantation to the injured part. Local administration of stem cells of SCI treatment can be done with intramedullary and intrathecal injection [45]. Several studies reported that intramedullary injections are more effective than intravenous injection to transplant stem cells to the injured area. They also reported that intrathecal injection is less invasive than intramedullary injection. The intrathecal injection also reduces the risk of rejecting stem cells by the host's immune system [49], [50].

A study by Levi _et al_. evaluated the safety of intramedullary injections to treat chronic cervical and thoracic SCI. They reported no adverse events associated with cell transplantation in cervical or thoracic SCI [51]. Study by Amemori _et al_. compared the effects of intramedullary and intrathecal transplantation of NSC on SCI in rats. They found that intramedullary injection provided more prolonged survival of cells for 2 months compared to intrathecal injection. Cells injected by intrathecal injection were reported absent at the administration site or in the spinal cord tissue. They also reported that intrathecal transplantation of stem cells might have a mild therapeutic effect on SCI through a paracrine mechanism. However, in chronic SCI, the longer survival time of intramedullary cells promotes regeneration of spinal cord tissue in the long term [52].

The ultrasound-guided injection can be used to assist in confirming which lamina needed to be removed to expose the post-traumatic cyst adequately. As we already know, the evidence of injury epicenter in the external or pial surface is subtle and indistinguishable. This technique helped define the caudal and rostral regions of the cyst so the appropriate location of injection could be determined [51]. Following transplantation, it was reported that ENSCs appeared to become progressively more dorsalized. This phenomenon causes almost exclusive localization of transplanted stem cells to the dorsal spinal cord [34]. The possible explanation for this may be caused by the presence of ventral neuroepithelial cells of the spinal cord. These neuroepithelial cells produce large populations

on the ventral part of the spinal cord, which places pressure on the overlying dorsal cells. Eventually, these neuroepithelial cells progressively forcing the transplanted stem cells more dorsally [53], [54].

In chronic SCI, the growth ability of axons through lesional tissue is significantly decreased [55]. The glial scar was developing through astrogliosis and forming well into the chronic stage of injury. Chondroitin sulfate proteoglycans (CSPGs) are elements of the extracellular matrix that formed the wall of the cystic cavity in chronic SCI. Removing them could significantly improve outcomes following cell transplantation because increasing growth ability of stem cell to penetrate the cavity [56].

The bacterial enzyme Chondroitinase ABC (ChABC) is known to have the ability to cleave CSPG moieties and degrades the impedance to plastic regeneration. A study reported that, when combined with NSC, ChABC leads to more significant cell migration and synaptic plasticity in SCI [57]. Another study also reported that combination of ChABC, NSPCs, and growth factor infusion increased transplanted cell migration, improved oligodendroglial remyelination, motor plasticity, and locomotor recovery [58]. Additional reports also suggested that ChABC can improve other treatment modalities and activate endogenous NSC [59], [60]. Simultaneous application of ENSCs and ChABC resulted in significant improvements in lesion histology compared to those of either treatment alone. Combined application of ChABC and ENSCs resulted in similar survival and spread of transplanted cells compared to transplant ENSCs-only. Further, dual treatments increased the number of retrograde axons crossing the injury site and reduced the amount of reactive astrogliosis in the injury zone [34].

Conclusion

Mesenteric NSC is a promising stem cell therapy to treat chronic SCI with low risk and easier procedure to isolate cell compare to other sources of NSC.

Author Contribution

All authors contribute equally in this article.

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