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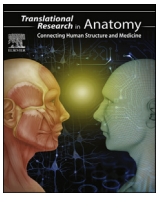

Review

Engraftment of neural stem cells in the treatment of spinal cord injury

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ABSTRACT

Spinal cord injury is one of the main causes of disability in the young population. Based on the underlying pathological changes, many modalities of treatments have been trialed. However, the most promising so far, has been the replacement of lost cellular elements, using stem cells and non-stem cells transplantation. The route of cellular administration and engraftment into the site of injury is an important determining factor for functional outcome, and should be chosen to be safe and efficacious in human patients. Herein, we will review the underlying changes following spinal cord injury, and the possible routes of cellular transplantation.

Contents

1. Introduction

In 1944, Woolsey et al. [1] reported the first spinal cord transplantation in known history. A 16- year-old male presented with

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complete loss of motor and sensory function after he was shot in his right shoulder with the bullet reaching the superior border of the fourth thoracic vertebra. Following laminectomy, the injured spinal cord was completely transected and replaced with a cadaveric spinal cord that had been fixed in 10% formalin for twelve days, and cleaned and sterilized with running and distilled water and 70% alcohol. No improvement in the patient's condition was noted, and the patient died almost 4 months after the surgery. Autopsy showed exceptional preservation of the transplanted graft, although with restricted regeneration and limited tissue reaction. The preservation was attributed to the preoperative use of formalin, and no explanations or related conclusion on the microscopic findings could be made.

2. Spinal cord injury

The world wide annual incidence of spinal cord injury (SCI) is 15–40 cases per million. The incidence is approximately 12,000 cases in the United States. Of these, 4000 die before reaching the hospital and 1000 during hospitalization, mostly due to pneumonia and septicemia [2]. Most of these injuries occur in otherwise healthy and young patients, and are mainly due to fracture and/or dislocation of the vertebral column [3]. Based on gross findings, SCI can be classified into four groups: (1) solid cord injury, the least common type, associated with normal appearance of the spinal cord after injury; (2) contusion/cavitation, the most common type, associated with areas of hemorrhage, and expanding necrosis and cavitation, but with no disruption of the surface of the spinal cord; (3) laceration, where there is a clear-cut disruption of the surface anatomy; and (4) massive compression, where the cord is macerated or pulpified to varying degrees. However, despite the differences in anatomic disruption of the spinal cord, these findings carry no significant differences in the consequent histological changes. This disparity is dependent on the different phases of SCI (see below), leading to progressively deteriorating neuronal function [4].

2.1. Pathological changes

Pathological changes following SCI can be divided into two, partially overlapping, phases: primary and secondary [3]. With more thorough analysis, four main phases also have been described: immediate hyperacute, acute, intermediate, and late phases [4]. In the following text, we will discuss these phases with focus on their effects on the neural cells, oligodendrocytes (OL), and oligodendrocytes progenitor cells (OPC), which are the main determinant of regeneration and cellular replacement therapy.

The immediate hyperacute phase is caused by the primary insult of injury, and usually takes place within the first 1–2 h of injury. During this phase, the initial insult, whether it is a contusion, compression, shearing, or stretching of the spinal cord, will lead to disruption of the neural and endothelial tissue. This is associated with hemorrhagic necrosis that is mainly localized in the gray matter and the center of the cord. The localization is due to the high vascularity of the gray matter and the epicentric movement of the injured tissues, which, in turn, places the most damage on the centrally located cells, and the least on the subpial ones. Moreover, at the site of injury, myelinated axons exhibit more pathological injury than unmyelinated ones. This is because the longitudinal force (especially in spinal cord contusion) stretching the fibers is concentrated at the nodes of Ranvier. In many cases, however, no abnormalities are seen following the initial trauma, and most of the consequent changes depend on more insidious, though devastating, secondary injury [3,4].

Following the first 3 h of injury, the secondary phase begins. This phase can be further divided into acute phase (hours to 3 days), intermediate (days to weeks), and late phase (weeks to months) [4]. However, as most of the processes that occur during the secondary injury are interconnecting, we do not prefer the use of this subdivision.

During the secondary injury, expansion of the hemorrhagic sites appears early, and is related to cellular death, which is precipitated, by acute necrosis and subacute apoptosis. Inflammatory response is an important determinant in this process. It starts during the first day of injury, and is initiated by the release of the chemical mediators that attract the early inflammatory cells (i.e. neutrophils) to the site of injury. Neutrophils release inflammatory mediators and free radicals that will exacerbate and accelerate the secondary phase of injury [4]. Necrosis starts as a wave that spreads in centripetal and rostro-caudal directions from the site of primary injury. This necrosis occurs via various mechanisms, including, infarction, excitotoxicity, and reperfusion injury [3,5].

Infarction, which begins during the primary injury phase, occurs early due to disruption of the vascular bed, which, in turn, interrupts the blood perfusion to the neural tissue, and leads to release of toxic digestive proteolytic enzymes. Thereafter, inflammatory changes associated with vasospasm, thrombosis, and neurogenic shock play important role during the secondary phase. The resulted hypoperfusion is associated with inhibition of both oxidative phosphorylation and glycolytic pathways, and leads to loss of energy production and consequent necrosis. *Reperfusion* of the site of injury during this stage will exacerbate cellular death. This is due to reactive oxygen species (ROS) formation from the ischemic endothelial cells. This, added to the ROS produced by the inflammatory cells, will cause direct damage and necrosis to the reperfused cells. *Excitotoxicity* is initiated by the accumulation of the glutamate within the extracellular spaces at the sites of injury. This accumulation is mainly due to defected absorption, excessive release from the damages cells, and exocytosis of the glutamate synaptic vesicles. Glutamate will then lead to over activation of the neural depolarization by activation of the glutamate receptors. Such persistent depolarization will create ionic and osmotic imbalance across the plasma membrane that will cause water influx and consequent lyses. It also leads to excessive calcium influx into the cell and the activation of the auto-destructive calcium-dependent enzymes [3]. Moreover, the release of glutamate and adenosine triphosphate (ATP) at the site of injury will activate the glutamate and P2X7 receptors, respectively, on the OL and OPC. These receptors attract the OL and OPC to the site of injury and cause further cellular loss in similar mechanism as described above [6].

Apoptosis begins as early as 6 h following injury, and spreads in a wave similar to that in necrosis. During the early phase, almost any cell type can be involved. Later on, the OL and myelinated cells are predominantly involved [3]. This programmed cell death occurs due to the secretion of inflammatory mediators and the extravasation of toxic substances following the injury [6]. Some authors, however, deny the presence of apoptosis during SCI in humans [4]. The above-mentioned processes, although extending through the following phases, comprise the main components of the acute phase of the secondary injury.

Over the ensuing days and weeks, more inflammatory cells will invade the site of injury in order to clear the debris and initiate the process of healing via neural fibrosis or gliosis. This starts with accumulation of the myelin and OL debris followed by activation and migration of microglia and macrophages which phagocyte these debris. At this early stage, the phagocytosis may enhance the regenerative process. Moreover, microglia may contribute, via the secretion of various cytokines including IL-1β, Il-6, and TNFα, to facilitate neural protection and regeneration. However, overtime,

progressive maturation of the glial scar, followed by migration and proliferation of the astrocytes, inhibits the regeneration and remyelination of the neuronal cells [3,4].

At the same time, in an attempt to maintain the viability of the remnant tissue and slow the progression of tissue loss, increased number of blood vessels can be noticed at the site of injury. This is mostly due to combination of tissue loss and preservation of the vascular structure, and the secretion of angiogenic factors in response to the inflammatory process [4].

Axonal disruption starts as early as few minutes following SCI, as described above. The periaxonal swelling leads to rupture and peeling of the surrounding myelin, which can be observed in the extracellular space 24 h after SCI. This process is accompanied by Wallerian degeneration (WD) which continues to progress for 1–22 years following the injury, and forms the major component of the late secondary injury. WD is mainly characterized by degeneration and disruption of the axonal function, and it usually extends in cranial and caudal directions from the initial site of injury. Moreover, in the long term, WD of the axons induces sustained apoptosis of the OL, which are supported by the trophic factors released from these axons. A few weeks after injury, these factors combined, if uninterrupted, will lead to widespread demyelination of the axons. However, this is usually prevented by concomitant remyelination, which may start few weeks after the insult [3,4,6,7].

Although remyelination is not perfect, it is sufficient to preserve function of spared intact axons, and maintain their integrity and function. The acute phase remyelination is mediated by the proliferating myelogenic progenitor cells that present at the margins of injury, and is identified by the expression of nerve/glial antigen 2 (NG2) or platelet derived growth factor receptor (PDGFR). Mature form of the OPC has less capacity to remyelinate, and needs prolonged exposure to growth factors to convert into proliferating cells. The specification of OL from the progenitor cells is induced by the sonic hedgehog (SHH) and opposed by the bone morphogenetic factor (BMP). Both SHH and BMP are up regulated at the site of injury. The presence of the astrocytes, often produced by the proliferating OPC, is essential. They play a role in maintaining the survival, proliferation and differentiation of the OPC and OL by secreting different types of growth factors during the early phase of spinal cord injury. However, with time, secretion of these factors will be decreased, which will lead to a progressive decline in the OL and OPC ability to remyelinate and maintain the axonal function. Moreover, despite the essential early role of the astrocytes, at the second to four weeks of injury, they begin to form a dense astrocytic scar surrounding the demyelinating axons. They may also express other molecules (e.g. Jagged1) that inhibit maturation and differentiation of the OL and OPC. Another type of scar, a mesenchymal scar, will also form by infiltrating fibroblasts and collagen fibers, stimulated by the injured glia limitans of the subpial space. These scars will prevent the OL and OPC from reaching the site of injury, limit the ability of injured nerve cells to regenerate, and form a therapeutic obstacle. Other late changes may include Schwannosis, in which the injured spinal tissues are replaced by Schwann cells. Cysts and syrinx formation may also be seen [4,6–8].

For these reasons, early treatment of spinal cord injury is crucial to enhance the locomotor function, and this window that extends from the acute inflammation to onset of the scar formation represents the 'therapeutic window'.

3. Functional deterioration

The functional deterioration after SCI can be classified according to the American Spinal Injury Association (ASIA) into complete (ASIA "A"), where there is no sensory or motor function below the level of injury; incomplete (ASIA "B," "C," or "D"), where sensory functions, with or without varying degree of motor functions, are lost below the level of injury; and ASIA "E", where the patient is functionally normal [9]. It is fundamental to note that it does not automatically infer that functionally complete injuries are anatomically complete, which is uncommon, and it can be explained by tissue sparing. Thus, even small preservation (~10–15%) and/or regeneration of the lost fibers may be enough to restore meaningful function, and this can be applied most effectively on individuals with functionally incomplete and some with complete injury [10]. Thus, beside the degree of injury and functional loss, it is vital to identify the degree of anatomically preserved fibers, and the site and extent of injury.

4. Cellular replacement and stem cells

Based on the above-mentioned pathological changes following injury, many methods of treatment have been applied to slow and reverse the progressive derangements. These include pharmacological and non-pharmacological methods [11–13]. Nevertheless, most of these treatment modalities have faced serious limitations, including the restricted capacity for regeneration and repair of damaged spinal nerve cells and tracts, and the limitation in neural plasticity. These also include the permanent neuronal loss and gap formation that complicate the SCI, and the extrinsic inhibition that adds on the intrinsic restricted regeneration [14].

To overcome these obstacles, replacement of the lost elements of the SCI, has gained most attention for clinical research, and has become the most promising method of treatment. The transplanted cells should enable regenerating axons to cross barriers, functionally replace lost cells, and/or create an environment supportive of neural repair [15]. These efforts are mostly directed towards white matter injury, which carry the biggest burden of the functional disability. However, regeneration of the gray matter has also an important role in restoring proprioception and muscle coordination [10]. Cellular and paracellular transplantation for SCI include stem cell and non-stem cell transplants. Non-stem cell transplants include olfactory ensheathing cells, Schwann cells, peripheral nerves, and genetically modified fibroblasts.

Table 1
Comparison between different routes of cellular engraftment.

Route	Advantage	Disadvantage
Intramedullary	- Most effective - Direct access to the site of injury	- Most invasive - Surgery-related complications - Multiple injections are often needed - Limited efficacy during chronic phase of injury
Intrathecal	- Less invasive - Effective	- Limited efficacy during chronic phase of injury
Intraventricular		- More invasive than intrathecal, with equal efficacy
Intravascular	- Least invasive	- Least effective

5. Methods of cellular engraftment

One of the most important factors in stem cell therapy is the route of cellular administration. In previous studies, different routes for cellular transplantation into the injured spinal cord have been trialed. However, the fact that most of the recipients were and still are animals with limited trials on humans, makes it difficult to compare the safety and effectiveness of these methods, and more studies on this field are still required. (Table 1).

5.1. Intramedullary route

Direct intramedullary engraftment represents a classical mode of cellular transplantation in animal models of SCI. This invasive method involves direct access to the site of injury via laminectomy followed by multiple injections of the transplant into the injury epicenter and/or into the parenchyma adjacent to the injury. This route has been applied on animals using different types of human stem cells, including neural stem cells of various origins [16–21], oligodendrocytes [22], motor neurons [23], and bone marrow stem cells [24]. The major disadvantage of this method is its invasiveness and the associated risk of causing further harm and trauma to the injured tissue during surgery, risking additional functional deterioration. This method may also compromise the vulnerability of cells, which are transplanted into the hostile environment of the injured spinal cord due to released inflammatory and cytotoxic chemokines [25]. Moreover, multiple injections at different points of time are needed, which is always associated with risk of complications due to anesthesia or the neurosurgical procedure [26]. During surgery, dura mater is often compromised rendering the patient more susceptible to postoperative CSF leak, in addition to other postoperative complications such as deep venous thrombosis and pulmonary complications [27]. All these factors make this route far from optimal for clinical application on human patients. However, there are some clinical trials, which have used this method on humans in different stages of SCI. The outcomes of these studies were very promising and considered superior by the authors as compared to other routes including intrathecal route, and with no remarkable complications [28,29]. This is especially true in cases of subacute and chronic SCI, and might be related to the limited migration and time window of recovery associated with the intrathecal engraftment. In light of the above-mentioned drawbacks, although still unproven over long term in human clinical trials, less invasive methods were investigated.

5.2. Intrathecal route

Intrathecal cellular transplantation via lumbar puncture (LP) was first introduced by De la Calle et al. [30] in 2002. Hence after, this technique was adopted as a minimally invasive method to deliver stem cell transplant into injured spinal cord by Bakshi et al. [31]. These authors, in this and later studies [25,32] used different types of cells including bone marrow stem cells and neural precursor cells. They reported cellular accumulation in large numbers at the site of injury, mainly at the interface of injury and meninges, following transplantation. Except for a few cells in the lining of brain ventricles, no other transplanted cells were noticed in intact neural tissues. This selective homing mechanism is mediated by chemotactic signals expressed at the injury site. These signals include SDF-1α and its CXCR4 receptor that are presented on the transplanted cells. Other factors may include platelet-derived growth factor (PDGF), transforming growth factor alpha (TGFα), insulin growth factor (IGF), and hepatocyte growth factor (HGF). This homing process appears to be more active and effective during early stage of injury, evident by more cellular accumulation occurring at this stage. Although more toxic substances and hostile environment are present at this time, the amount of secreted chemokines involved in attracting cells to the injury is increased in the initial phase. Moreover, the healing process associated with glial scar formation will limit the cellular migration and integration at later points of time. Thus, according to the authors, the window of opportunity for intrathecal delivery, is limited to the acute and partly the subacute phase of SCI, and not optimal at the chronic level, unless glial scar debridement was initiated first [26].

This window of opportunity was proven in clinical trials on human patients where intrathecal engraftment showed minimal functional improvement in patients with subacute and early chronic SCI (<6 months), but failed to show any improvement in patients with late chronic SCI (>6 months) [33–36].

When comparing this mode of cellular transplantation with the direct intramedullary injection on animal models with acute and subacute phases of SCI, functional improvement were more remarkable using the latter, and that was even more noticeable in chronic phase injury. Both methods, however, were neuroprotective, resulting in reduction of injury size and greater tissue sparing, in addition to better functional outcomes compared with controls [37].

Although this represents a less effective method so far, it limits patient risk, side effects, and cost and can be used to deliver multiple doses of cells. In regard to its limitation in advanced phases of SCI, it is believed that optimization of the LP procedure in the future by further optimization of cell dosage, timing of delivery, and number of deliveries may improve grafting efficiency and thereby functional recovery to levels comparable to direct injection [26].

5.3. Intraventricular route

Stem cells engraftment for SCI through the ventricular system of the brain was once a favored method of cellular replacement [38]. However, with the development of more effective and minimally invasive modes of delivery, it has been almost abandoned. This method includes direct injection of the transplant cells into a ventricular cavity, followed by cellular migration and integration at the site of injury in the same homing mechanism as the intrathecal route. Although these two routes have comparable functional outcomes, the latter is much less invasive and more reliable for clinical applications [39,40].

5.4. Intravascular route

The systemic delivery of the transplanted cells via intravascular route (intra-arterial or intravenous) represents the least invasive, though the least efficacious, method of engraftment. The multisegmental arterial supply to the spinal cord limits the use of intra-arterial delivery, as it requires highly selective and technically challenging cannulation of the spinal arteries [17]. On the other hand, intravenous delivery is a safer and easier method to apply. Experimental trials on animal models with SCI using intravenous route have shown promising results [41] with evidences of cellular migration to the site of injury mediated by HGF and stromal cell-derived factor-1 (SDF-1), which peaks at day 7 of injury [42]. Nevertheless, the undisrupted blood–brain barrier (BBB) still presents a limiting factor in the effectiveness of this route. Additional limiting factors include the first-pass effects and trapping of these cells in extraneural tissues such as lung and liver, along with the prolonged exposure to the immune cells during circulation [27,31]. Although the number of cells accumulating at the site of injury increases with time and associated with mild functional improvement, most studies have reported markedly decreased engraftment efficiencies as compared to other routes of delivery, keeping in

mind that as time passes more irreversible neural degeneration is expected [27,31,43,44]. Using this route in human patients has proven some degrees of functional recovery that was mainly consistent in patients with acute and subacute phase injury, and much less effective in chronic phase. That gives this route the same window of opportunity as the intrathecal one [45,46].

6. Clinical trials on humans

Clinical trials on patients with SCI using stem cells are very limited due to the lack of sufficient evidences on effectiveness. Several factors were shown to affect the success of treatment, including time of intervention, source of stem cells, and route of administration. As discussed above, the earlier the intervention during acute phase of injury, the better the outcome. Yoon et al. [47] used intramedullary rout for administration of autologous bone marrow cell in patients with acute (up to 2 weeks), subacute (2–8 weeks), and chronic (more than 8 weeks) SCI. Over 10 months of follow-up, noticeable locomotor improvement was noted in the acute and subacute patients to variable degrees, but none in the chronic patients. No permanent or serious complications were reported.

Two sources of stem cells have been mainly used for treatment of patients with SCI, including autologous bone marrow stem cells, and mesenchymal stem cells derived from either bone marrow or umbilical cord [48]. Treatment using whole autologous bone marrow stem cells rather than only mesenchymal stem cells has shown more promising results [28,29,33,35,47–49]. Nevertheless, studies with bone marrow stem cells where often performed during acute phase of injury, compared to chronic phase in studies using mesenchymal stem cells. Moreover, some studies with bone marrow stem cells have added subcutaneous injections of granulocyte macrophage-colony stimulating factor, which was found to have direct effect on the transplanted BMC by enhancing their survival in the spinal cord and activating them to excrete neurotrophic cytokines [29,47].

Although intramedullary route was reported superior for stem cell delivery in animal studies (see above), no significant difference in outcomes and complications was noted by Geffner et al. [43] comparing intramedullary, intrathecal, and intravascular routes in patients with SCI. Both intramedullary and intrathecal administration have also been combined for better results in chronic SCI [49]. Therefore, further human trials are needed for more conclusive results.

7. Conclusion

Cellular transplantation has become the most promising treatment modality for SCI. Overtime, several animal-based studies have been conducted to assess the efficacy and safety of this treatment before it could be widely used in humans. One of the important determining factors in cellular transplantation is the route of cellular administration. Three main routes have been used in most studies, which are, in order of efficacy, the intramedullary, intrathecal, and intravascular routes.

Conflict of interest statement

The authors declare that they do not have any conflict of interest.

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