Current Status and Future Strategies to Treat Spinal Cord Injury with Adult Stem Cells

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Spinal cord injury (SCI) is one of the most devastating conditions and many SCI patients suffer neurological sequelae. Stem cell therapies are expected to be beneficial for many patients with central nervous system injuries, including SCI. Adult stem cells (ASCs) are not associated with the risks which embryonic stem cells have such as malignant transformation, or ethical problems, and can be obtained relatively easily. Consequently, many researchers are currently studying the effects of ASCs in clinical trials. The environment of transplanted cells applied in the injured spinal cord differs between the phases of SCI; therefore, many researchers have investigated these phases to determine the optimal time window for stem cell therapy in animals. In addition, the results of clinical trials should be evaluated according to the phase in which stem cells are transplanted. In general, the subacute phase is considered to be optimal for stem cell transplantation. Among various candidates of transplantable ASCs, mesenchymal stem cells (MSCs) are most widely studied due to their clinical safety. MSCs are also less immunogenic than neural stem/progenitor cells and consequently immunosuppressants are rarely required. Attempts have been made to enhance the effects of stem cells using scaffolds, trophic factors, cytokines, and other drugs in animal and/or human clinical studies. Over the past decade, several clinical trials have suggested that transplantation of MSCs into the injured spinal cord elicits therapeutic effects on SCI and is safe; however, the clinical effects are limited at present. Therefore, new therapeutic agents, such as genetically enhanced stem cells which effectively secrete neurotrophic factors or cytokines, must be developed based on the safety of pure MSCs.

Key Words : Adult stem cells · Spinal cord injuries · Mesenchymal stem cells · Neural stem cells · Genetic enhancement · Stem cell transplantation.

INTRODUCTION

Spinal cord injury (SCI) is one of the most devastating conditions in the neurosurgical field. Even with the best medical, surgical, and rehabilitative treatments, many SCI patients suffer neurological sequelae. Multiple strategies have been ap-plied to treat SCI patients, such as pharmacological treatment, neuromodulation, and surgical trials. Cell transplantation is considered to be a promising strategy due to the limited efficacies of other approaches. Among the many candidates for transplantable cells, embryonic stem cells (ESCs) have gained attention due to their capacities to differentiate into nearly all

human tissues and to undergo unlimited expansion in vitro, and thereby generate the required number of cells[20]. However, although ESCs have advantages over adult stem cells (ASCs) in terms of their accessibility, expansion, and differentiation[55], they carry the risk of tumorigenesis and are associated with ethical problems[3,54]. Consequently, clinical interest in ASCs has grown. Here, we reviewed and summarized the current status of the development of ASCs therapies for SCI and described several future strategies to treat SCI using ASCs.

STRATEGY OF STEM CELL THERAPY FOR SCI

Spontaneous healing mechanisms, including remyelination, neural plasticity, and endogenous stem cell activation, are activated after SCI. However, these mechanisms are insufficient to produce clinically significant functional recovery in humans[33]. The tissue targets in SCI are mainly neuronal axons not neuronal bodies, and the target space is much smaller than the brain. Cellular treatment in SCI has several goals : re-

ducing cell death and damage due to the secondary injury in the immediate phase after SCI, promoting axonal regeneration and tissue repair in the late phase[36]. Stem cells facilitate spinal cord repair in the chronic phase via various mechanisms, such as enhancing remyelination of residual axons, secreting neurotrophic factors/cytokines, producing bridging materials to fill cavities formed upon SCI, resolving intramedullary cavity margins composed of glial scars[37,41], and activating endogenous intramedullary stem cells[50]. Other mechanisms, which are promoting angiogenesis and reducing inflammation, may also be important in the acute phase of SCI[36].

Recently, an animal study was conducted based on the hypothesis that the central pattern generator (CPG) contributes to functional restoration in SCI upon stem cell therapy[52]. Within the spinal cord of mammals and other vertebrates, a neuronal circuit, called the CPG, is thought to generate repetitive motor patterns related to walking, running, and scratching. The CPG is known to be located in the L2 segment of the spinal cord in humans[65].

Table 1. Clinical trials of stem cell transplantation in the subacute phase of SCI

Study	Stem cell type	Tx. time from SCI	SCI site	PreTx. AIS	N	PostTx. AIS	N
Shin et al.[47] (2015)	NSPCs	63.4±54.1 (18–213) days	Cervical	A	17	A→A	14
						A→B	1
						A→C	2
				B	2	B→D	2
Anderson et al.[2] (2017)	SCs	40.2±11.3 (29–59) days	Thoracic	A	6	A→A	5
						A→B	1
Saito et al.[45] (2012)	BM-MSCs	13.0±2.9 (8–17) days	Cervical	A	3	A→A	3
				B	1	B→D	1
				C	1	C→D	1
Karamouzian et al.[23] (2012)	BM-MNCs	27.3±8.4 (14–43) days	Thoracic	A	11	A→A	6
						A→C	5
Park et al.[40] (2005)	BM-MCP+GM-CSF	9.8±2.9 (7–14) days	Cervical	A	4	A→A	1
						A→B	1
						A→C	2
		8 days	Thoracic	A	1	A→C	1
Knoller et al.[27] (2005)	Activated autologous macrophages	12.3±1.9 (9–14) days	Cervical	A	1	A→C	1
			Thoracic	A	7	A→A	5
						A→C	2

SCI : spinal cord injury, Tx. : treatment, AIS : American Spinal Injury Association Impairment Scale, N : number of patients, NSPC : neural stem/progenitor cell, SC : Schwann cell, BM : bone marrow, MSC : mesenchymal stem cell, MNC : mononuclear cell, MCP : mononuclear cell preparation, GM-CSF : granulocyte-macrophage colony-stimulating factor

TIME WINDOWS FOR STEM CELL THERAPY IN SCI

According to the guidelines issued by the International Campaign for Cures of SCI Paralysis (ICCP) panel, the acute phase of SCI is defined as the period until the third day after injury, while the chronic phase is defined as the status after 12 months of injury with no neurological changes in the previous 6 months[12]. Consequently, the subacute phase has a broad time window. The environments of transplanted cells in the injured spinal cord differ according to the phases of SCI. Therefore, many researchers have investigated these phases to determine the optimal time window for stem cell therapy in

animals[54]. In addition, the results of clinical trials should be evaluated according to the phase in which stem cells are transplanted to determine the optimal time window (Tables 1-3).

In animal studies, the subacute phase is generally considered to be optimal for stem cell transplantation[36,54]. In this phase, glial scar formation is less advanced and the inflammatory response has subsided. Transplantation of stem cells in the subacute phase can prevent secondary injuries. In addition, transplanted stem cells survive better in the subacute phase than in the acute phase[38]. However, clinical trials of stem cell transplantation in the subacute phase require the enrollment of larger numbers of patients for case-control cohorts compared to the chronic phase which does not necessarily re-

Table 2. Clinical trials of stem cell transplantation in the chronic phase of SCI

Study	Stem cell type	Tx. time from SCI	SCI site	PreTx. AIS	N	PostTx. AIS	N
Zhu et al.[67] (2016)	UCB-MNCs	8.8±6.2 (1.6–20.0) years	Cervical	A	8	A→A	7
						A→B	1
				C	1	C→C	1
			Thoracic	A	19	A→A	14
						A→B	2
						A→C	3
Vaquero et al.[59] (2016)	BM-MSCs	13.9±9.4 (3.2–26.8) years	Thoracic	A	12	A→A	8
						A→B	3
						A→C	1
Oh et al.[37] (2016)	BM-MSCs	5.2±2.8 (2.0–15.1) years	Cervical	A	1	A→A	1
				B	15	B→B	15
Al-Zoubi et al.[1] (2014)	Autologous, purified CD34+ and CD133+ stem cells	1.7±0.8 (1.0–4.0) years	Thoracic	A	19	A→A	10
						A→B	7
						A→C	2
Mendonça et al.[34] (2014)	BM-MSCs	5.1±4.1 (1.5–15.0) years	Thoracic	A	14	A→A	5
						A→B	6
						A→C	1
Kishk et al.[26] (2010)	BM-MSCs	3.6±2.5 years	Cervical, thoracic	A	40	A→A	28
						A→B	11
						A→C	1
			Thoracic	C	3	C→C	3
Deda et al.[8] (2008)	BM MCP	5.1±5.0 (2–17) years	Cervical, thoracic	A	9	A→B	2
Saberi et al.[44] (2008)	SCs	3.9±1.7 (2.3–6.7) years	Thoracic	A	2	A→A	2
				C	2	C→C	1
						C→D	1

SCI : spinal cord injury, Tx. : treatment, AIS : American Spinal Injury Association Impairment Scale, N : number of patients, UCB : umbilical cord blood, MNC : mononuclear cell, BM : bone marrow, MSC : mesenchymal stem cell, MCP : mononuclear cell preparation, SC : Schwann cell

Table 3. Clinical trials of stem cell transplantation in the mixed phase of SCI

Study	Stem cell type	Tx. time from SCI	SCI site	PreTx. AIS	N	PostTx. AIS	N
Hur et al.[19] (2016)	AD-MSCs	17.4±6.2 (12–28) months	Cervical	A	2	A→A	2
				D	1	D→D	1
			Thoracic	A	5	A→A	5
			Lumbar	A	1	A→A	1
		7.0±3.3 (3–11) months	Cervical	A	3	A→A	3
				B	1	B→B	1
			Thoracic	A	1	A→A	1
Bhanot et al.[4] (2011)	BM-MSCs	46.3±36.7 (18–132) months	Cervical	A	4	A→A	4
			Thoracic	A	7	A→A	6
						A→B	1
		6.8±2.7 (3–10) months	Cervical	A	2	A→A	2
			Thoracic	A	4	A→A	4
Pal et al.[39] (2009)	BM-MSCs	>6 months	Thoracic	A	9	A→A	9
				C	1	C→C	1
		<6 months	Cervical	A	6	A→A	2
				C	1	C→C	1
			Thoracic	A	9	A→A	9
				C	4	C→C	3
Geffner et al.[14] (2008)	BM-MCP	10.2±6.7 (5.8–21.8) years	Thoracic	A	1	A→C	1
				B	1	B→C	1
				C	2	C→C	1
						C→D	1
		5 days to 7 months	Thoracic	A	4	A→A	1
						A→C	3
Yoon et al.[64] (2007)	BM-MNCs	<2 weeks	Cervical, thoracic	A	17	A→A	12
						A→B or A→C	5
		2–8 weeks	Cervical, thoracic	A	6	A→A	4
						A→B or A→C	2
		>8 weeks	Cervical, thoracic	A	12	A→A	12
Syková et al.[51] (2006)	BM MCP	18.0±2.9 (15–22) months	Cervical	B	1	B→B	1
				C	1	C→C	1
			Thoracic	A	1	A→A	1
		10 days to 9 months	Cervical	A	7	A→A	6
						A→B	1
				B	3	B→B	2
						B→B	1
			Thoracic	A	7	A→A	7
Lima et al.[29] (2006)	OECs	44.4±18.0 (30–78) months	Cervical	A	2	A→A	1
						A→C	1
			Thoracic	A	3	A→A	3
		6 months	Cervical	A	1	A→A	1
			Thoracic	A	1	A→C	1

SCI : spinal cord injury, Tx. : treatment, AIS : American Spinal Injury Association Impairment Scale, N : number of patients, AD : adipose tissue-derived, MSC : mesenchymal stem cell, BM : bone marrow, MCP : mononuclear cell preparation, MNC : mononuclear cell, OEC : olfactory ensheathing cell

quire double-arm study[37].

Tables 1-3 show the results of clinical trials using ASC transplantation (including one activated macrophage and two Schwann cell trials) in various phases of SCI according to the American Spinal Injury Association Impairment Scale (AIS). Although many SCI patients treated with ASCs showed no changes in terms of AIS, some studies reported improvements of two AIS grades (A→C or B→D)[1,14,23,26,27,34,40,45,47,51,59,64,67]. In addition, functional improvements such as walking[1,14,23,39,40,45,67] and bladder/bowel control[1,14,19,26,27,34,39,45,59,67] were used to reveal clinical change in several trials. It is challenging to directly compare the results of clinical trials between the subacute and chronic phases of SCI due to differences in the study design, population, and stem cell type. We could not determine whether stem cell transplantation in a certain phase leads to better clinical outcomes than in another phase based on the limited number of clinical trials presented in Table 3.

SAFETY OF ASCS

When transplanting cells into the human spinal cord, the most important issues are safety and complications. Safety and complication issues can be evoked by delivering procedures, malignant potential, immunogenicity of transplanted cells, or medical condition of SCI patients. In most of the clinical trials presented in Tables 1-3, researchers reported that their clinical trials were safe without mortalities or severe morbidities related to either procedures or transplanted cells. However, there were some reported complications which were not associated with the procedures or applied cells : fever[4,40,59,64], urinary tract infection[2,19,27,59], abnormal blood profiles[2,27,40,45], transient hypertension[26], vomiting[4,19], pulmonary thromboembolism[27], and general body ache[4]. On the contrary, some complications including transient neuropathic pain[2,26,27,29,37,67], transient deterioration in sensorimotor symptoms[29,37,44,64], cerebrospinal fluid leakage[67], subarachnoid hemorrhage[67] and subcutaneous seroma[67], might be related to stem cell delivering procedures. Herein, we focused on the safety of stem cell themselves with respect to their malignant potential and immunogenic properties.

Olfactory ensheathing cells (OECs) or olfactory ensheathing glia (OEGs) are stem cells in the olfactory system. The OECs/OEGs, the dominant glia in the peripheral nervous system, are known to guide axons along a defined path[58]. Given that OECs/OEGs can guide and ensheath axons, they are considered to be useful candidates for regeneration of the injured axon in spinal cord[62]. Although some studies reported that OECs/OEGs can be safely transplanted, the concerns about their safety still remain. In a human case report, intramedullary injected OECs/OEGs developed into a tumor when applied to treat chronic SCI[11]. Consequently, the safety of OECs/OEGs is controversial.

Dental papillary stem cells or dental pulp stem cells (DPSCs) are another source of ASCs. These cells can be harvested relatively easily and readily from discarded human teeth, such as wisdom teeth. However, an animal study in which human DPSCs were injected into rat brains reported that all rats died within 2 weeks of transplantation due to malignant brain tumor formation[63]. It is unclear whether DPSCs transformed into malignant tumor cells due to their inherent properties or due to the culture environment. No human clinical trial of DPSCs in SCI patients has been registered at ClinicalTrials. gov.

Transplanted Schwann cells (SCs) alter the inhibitory glial environment and induce axonal regeneration in SCI[22]. SCs are safe and there are no reports of malignant transformation or any other significant complications with these cells[2]. However, it is challenging to isolate SCs due to the risk of damaging other peripheral nerve segments, resulting in undesirable iatrogenic injury at the donor site[60]. New approaches to obtain SCs, such as differentiating these cells from bone marrow-derived mesenchymal stem cells (BM-MSCs)[24], umbilical cord-derived mesenchymal stem cells (UC-MSCs)[32], and adipose tissue-derived mesenchymal stem cells (AD-MSCs)[9], were recently reported. These approaches may be safe options for cell therapy of SCI.

MSCs can be obtained from various sources, including bone marrow, umbilical cord, and adipose tissue, without ethical concerns[25]. MSCs are of the mesodermal lineage, but can transform into ectodermal and endodermal lineages[57]. BM-MSCs are well studied and have relatively low immunogenicity[14]. In addition, these cells have never been reported to form malignant tumors in clinical trials[28,57]. Thus, BM-MSCs have been studied as ideal candidates for stem cell therapy for a long time. UC-MSCs can be obtained from various sources, but are most commonly isolated from Wharton's jelly[10]. Animal studies suggested that UC-MSCs elicit anti-cancer effects

on breast and lung cancer[7]. AD-MSCs are more useful than BM-MSCs and UC-MSCs in terms of abundance and accessibility[21]. However, there are fewer studies of AD-MSCs than of BM-MSCs and UC-MSCs.

Neural stem/progenitor cells (NSPCs) are multipotent cells that have self-renewing capacity and are destinated to differentiate into multiple neural lineages including neurons, oligodendrocytes, and astrocytes[36]. NSPCs have capacity to replace lost tissues and provide trophic support at the injured sites and are expected to reconnect neuronal circuits in the injured spinal cords[13]. Human NSPCs can be obtained from fetal or adult brain, and they can be also derived from ESCs and induced pluripotent stem cells. By developing alternative methods to obtain NSPCs, the ethical concerns by using human brain or ESC can be resolved, however, there is still the probability of tumorigenesis[16]. The immunogenicity of NSPCs is still debated[18,68].

COMPARISON OF MSCS AND NSPCS

ASCs candidates for treating SCI can be divided into two groups : MSCs (such as BM-MSCs, UC-MSCs, and AD-MSCs) and NSPCs. The major differences between these two groups of cells are the mechanisms by which they create therapeutic effects on SCI. In addition, their immunogenicity could be another different characteristic, which determines whether immunosuppressants are required or not.

MSCs do not usually differentiate into neural cells when injected into the spinal cord. However, many studies reported that transplantation of MSCs into the injured spinal cord exert functional improvement[4,10,19,23,26,34,41,43]. Injected MSCs are thought to indirectly affect axonal regeneration. The suggested underlying mechanisms include activation of endogenous stem cells via the production of cytokines[43], removal of glial scars[41], and formation of extracellular matrix which fills cavities and guides regenerating axons[37].

The rationale behind the use of NSPCs to treat SCI is based on replacement of destroyed tissue and provision of trophic support to surviving neuronal tissue[36,52]. Indeed, neural stem cells secrete several neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial cell-derived neurotrophic factor (GDNF)[30,49]. NPSCs are known to have less but no negligible immunogenic properties,

and immunosuppression is believed to be essential for transplanted cell survival due to possibilities of low-grade rejection[56]. In a clinical trial that transplanted human NSPCs into subacute SCI patients, immunosuppression was performed from 3 days prior to transplantation until 9 weeks after transplantation[47]. In a recent clinical study in which human NSPCs were transplanted into chronic cervical SCI patients, all subjects received immunosuppression regimens prior to engraftment and these were continued until 6 months post-transplantation to prevent graft rejection and mitigate any potential post-transplantation inflammatory responses[15].

Genetic modification of the MSCs may be a future tactic to improve therapeutic efficacy based on the safety of MSCs[46]. Our previous studies[37,41] demonstrated that BM-MSCs can be safely transplanted and affect axonal regeneration, but their efficacy is insufficient. To overcome this drawback of pure MSCs, genetically enhanced MSCs must be developed.

ARE ADDITIVE THERAPIES INCORPORATING STEM CELLS PROMISING?

Animal and/or human clinical studies have sought to enhance the effects of stem cells using combined scaffolds, trophic factors[42], cytokines[35,64], and other drugs[67].

Various animal studies investigated the effects of treatment with MSCs[17,66] and NSPCs[5,53] in combination with several scaffolds (poly lactic-co-glycolic acid, collagen, chitosan, gelatin, and hydroxypropyl methacrylate hydrogel with a Arg-Gly-Asp modification). These combination therapies improved axonal regeneration, stem cell differentiation, and functional improvement, and decreased scar formation[6,31,48]. In a recent human clinical trial, combination treatment with a collagen scaffold and UC-MSCs in the acute phase of SCI improved the AIS grade from A to C in one patient with cervical SCI and one patient with thoracic SCI after 1 year of follow-up[61].

Another method to enhance the effects of transplanted stem cells is to deliver them in combination with trophic factors. Animal studies in which stem cells (BM-MSCs, UC-MSCs, OECs, NSPCs, and SCs) were administered in combination with trophic factors (NT3, BDNF, cyclic adenosine monophosphate, and fibroblast growth factor) could not conclusively clarify synergistic effects[49]. In our previous study, we

also did not observe any additive effects when AD-MSCs were administered in combination with granulocyte colony-stimulating factor in rats with acute SCI[35].

In a clinical trial, treatment with autologous BM-MSCs in combination with granulocyte-macrophage colony-stimulating factor improved the AIS grade from A to B or from A to C in the acute and subacute phases of SCI, but did not induce any improvement in the chronic phase. However, this study did not include a control group using BM-MSCs only. In this regard, it is difficult to assess whether there was a synergistic effect[64]. Another clinical trial of 28 SCI patients reported that combination treatment with UC-MSCs and lithium improved the AIS grade from A to C in three patients and from A to B in two patients; however, this study also did not include a control group using UC-MSCs only, and failed to show the synergistic effect of combined therapy[67]. Lithium stimulates secretion of NGF, NT3, and GDNF by UC-MSCs.

CONCLUSIONS

The strategy for stem cell application on SCI is distinct from other central nervous system pathologies and there are different characteristics in mechanisms of action and immunogenicity among ASCs. Over the past decade, several clinical trials have suggested that transplantation of MSCs into the injured spinal cord elicits therapeutic effects and is safe. However, the clinical efficacy remains limited. Therefore, new therapeutic stem cell agents, such as genetically enhanced stem cells that effectively secrete neurotrophic factors or cytokines, must be developed based on the safety of pure MSCs.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

INFORMED CONSENT

This type of study does not require informed consent.

AUTHOR CONTRIBUTIONS

Conceptualization : SRJ
Data curation : SKJ, IC
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