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CASE REPORT

Case report on the clinical results of a combined cellular therapy for chronic spinal cord injured patients

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Objectives: With the intention to ameliorate the clinical condition of patients with chronic spinal cord injury (SCI), a program that combines three cell therapies and an appropriate neurorehabilitation program were used to recreate and enhance the natural conditions of SCI repair.
Methods: Vascularization recovery is approached by selective artery infusion of BMMNCs (bone marrow mononuclear cells) to the disrupted area. Eighteen days later, with the aim to restore the specific inflammatory activity, an i.v. infusion of spinal cord specific ETCs (effector T cells) is carried out. With the intention of supplying cellular components for the process of repair, an infusion of autologous neural stem cells (NSCs) through selective feeding artery infusion is carried out, followed by an appropriate neurorehabilitation program.
Results: A total of eight ASIA (American Spinal Injury Association) A patients (five with jeopardized brachial plexus and three without) received the treatment. No severe adverse events was observed in any of the receptor patients: five patients evolved from ASIA A to ASIA D and regained the ability to stand up and, with varying effectiveness, to walk; two patients remained in the same condition, but exhibited motor and sensitive improvements; and one patient could not be evaluated.
Conclusions: These reports suggest that the biological characteristics of acute SCI may be recreated in a comprehensive, safe and effective manner.
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Keywords: chronic spinal cord injury; neural stem cell; protective autoimmunity; neurorehabilitation; cellular therapy; scale evaluation

Introduction

The state-of-the-art treatment for chronic spinal cord injury (SCI) may achieve minor neurological function recovery in only $<5\%$ of the patients.[1]

This report describes the clinical results of three combined cell therapies associated with an appropriate neurorehabilitation program, which intends to recreate and enhance the natural conditions of SCI repair as described earlier.[2–5] The vascularization recovery is approached by selective artery infusion of BMMNCs (bone marrow mononuclear cells) to the disrupted area.[2] Eighteen days later, with the aim to restore the specific inflammatory activity,[3,4] a second cellular therapy is carried out by an i.v. infusion of spinal cord specific ETCs (effector T cells). It is carried out with the intention of opening the blood–brain barrier and for generating a neutropin microenvironment suitable for NSC repair.[3–5] The third cell therapy, infusion of autologous NSC through selective feeding artery infusion, is carried out with the intention of supplying the cellular components for the repairing process.[2,3] Finally, an *ad hoc* neurorehabilitation program was designed with the intention to procure an appropriate NSC spatial distribution to restore neural centers and brainstems. This sequence of cellular therapies, BMMNC-ETC-NSC, was named BEN.[2]

Adverse events were evaluated by the 'Common terminology criteria for Adverse Events (AE), 2004' developed by the NIH. See definitions on the web at http://ctep.cancer.gov/reporting/ctc.html.

Results

Treatment safety

After bone marrow puncture, 3/8 patients presented degree 2 anemia and 1/8 patients presented degree 1 headache and sickness.

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Table 1 Quantitative assessment scale of treated patients

Motor activity	*Definition*	*Intervening muscles*		*Joint movement*	*Spinal cord level*
Cephalic support	Active voluntary contraction of neck muscles	Sternohyoid, omohyoid, geniohyoid, digastric, scaleni, rectus capitis		Flexion, extension, rotation and tilp	C1–C5
Static trunk stability	Active and sustained contraction of paravertebral and abdominal muscles that allow trunk stability, at a 90° position with external assistance	Trapeziums, romboideus, deltoid, latissimus dorsi, iliocostalis, longissimus, quadratus lumborum, rectus abdominis, internal and external obliques, serratus muscle			
Dynamic trunk stability	Active and sustained contraction of paravertebral and abdominal muscles that allow trunk stability during lateral and antero-posterior movement, without external assistance			Flexion, extension, rotation and lateral tilp	C5–L1
Upper limb mobility	Active voluntary contraction	Shoulder	Deltoid, major and minor pectoral, latissimus dorsi, supraspinatus, infraespinous, teres major, teres minor, subscapularis	Raising, descending, adduction, abduction, internal and external rotation	C5–C8
		Elbow	Biceps, anterior brachial, brachioradialis, triceps, supinator, pronator	Flexion, extension, supination, pronation	C4–C7
		Wrist	Major and minor palmoris, anterior and posterior lunar, extensor carpi radialis longus	Flexion, extension, adduction and abduction	C6–C7
		Hand	Interoseus, lumbricals, extensor digitorum, thenar eminence muscles	Flexion, extension, thumb opposition, open and close in a fan manner	C8–D1
Pelvis mobility	Active contraction of agonist and antagonist muscles that allows a neutral position of orthostatism without the use of external support	Quadratus lumborum, rectus abdominis, internal and external obliques, transversus abdominis, longissimus, gluteus maximus and minimus, pelvic floor muscles			T7–L4
Pelvic floor contraction	Voluntary contraction of perineo muscles	Coccygeus, superficial transversus perinei muscle, levator ani, anal sphincter			S2–S4
Lower limb mobility	Voluntary active contraction	Hip	Psoas iliacus, gluteus maximus, medius and minimus, adductor magnus, longus and brevis, internus and externus obturator	Flexion, extension, adduction and abduction; internal and external rotation	L2–S1
		Knee	Quadriceps, crureus biceps, semimembranosus, semitendinosus, tensor muscle	Flexion, extension	L3–S2
		Ankle	Gastrocnemius, soleus, lateral penoneal, tibialis posterior and anterior, interoseus	Flexion, extension; adduction and abduction; internal and external rotation	L5–S2
		Foot	Finger flexors and extensor digitorum longus, inteoseus	Flexion, extension, finger abduction and adduction	L4–S1

Table 2 (a) Initial evaluation and (b) current evaluation

Pat.	*Cephalic support*	*Static trunk stability*	*Dynamic trunk stability*	*Upper limb mobility*			*Pelvis stability*	*Pelvis floor contraction*	*Lower limb mobility*			*Total*
					Left	*Right*				*Left*	*Right*	
(a)												
1	2	0	0	Shoulder	0	0	0	0	Hip	0	0	2/105
				Elbow	0	0			Knee	0	0	
				Wrist	0	0			Ankle	0	0	
				Hand	0	0			Foot	0	0	
2	4	0	0	Shoulder	2	3	0	0	Hip	0	0	11/105
				Elbow	0	2			Knee	0	0	
				Wrist	0	0			Ankle	0	0	
				Hand	0	0			Foot	0	0	
3	6	0	0	Shoulder	2	3	0	0	Hip	0	0	13/105
				Elbow	0	2			Knee	0	0	
				Wrist	0	0			Ankle	0	0	
				Hand	0	0			Foot	0	0	
4	6	3	0	Shoulder	5	5	0	0	Hip	0	0	49/105
				Elbow	5	5			Knee	0	0	
				Wrist	5	5			Ankle	0	0	
				Hand	5	5			Foot	0	0	
5	6	2	0	Shoulder	4	4	0	0	Hip	0	0	34/105
				Elbow	4	4			Knee	0	0	
				Wrist	3	3			Ankle	0	0	
				Hand	2	2			Foot	0	0	
6	6	4	3	Shoulder	5	5	1	0	Hip	0	0	53/105
				Elbow	5	5			Knee	0	0	
				Wrist	5	5			Ankle	0	0	
				Hand	5	5			Foot	0	0	
(b)												
1 8 m of treat.	4	2	0	Shoulder	3	4	0	4	Hip	0	0	23/105
				Elbow	3	3			Knee	0	0	
				Wrist	0	0			Ankle	0	0	
				Hand	0	0			Foot	0	0	
2 12 m of treat.	5	3	2	Shoulder	4	4	0	4	Hip	0	0	37/105
				Elbow	4	4			Knee	0	0	
				Wrist	2	3			Ankle	0	0	
				Hand	1	1			Foot	0	0	
3 24 m of treat.	5	5	3	Shoulder	4	4	2	4	Hip	2	2	54/105
				Elbow	4	4			Knee	0	0	
				Wrist	4	4			Ankle	0	0	
				Hand	3	3			Foot	0	0	
4 6 m of treat.	5	5	5	Shoulder	5	5	4	5	Hip	3	3	82/105
				Elbow	5	5			Knee	1	0	
				Wrist	5	5			Ankle	0	0	
				Hand	5	5			Foot	0	0	
5 5 m of treat.	5	3	2	Shoulder	5	5	2	2	Hip	0	0	53/105
				Elbow	5	5			Knee	0	0	
				Wrist	5	5			Ankle	0	0	
				Hand	3	3			Foot	0	0	
6 6 m of treat.	5	5	4	Shoulder	5	5	4	Not evaluated	Hip	4	4	68/105
				Elbow	5	5			Knee	1	1	
				Wrist	5	5			Ankle	0	0	
				Hand	5	5			Foot	0	0	

Abbreviations: Pat., patient; treat.,treatment.

During the apheresis procedure, 1/8 patients presented degree 1 arterial hypertension.

After the apheresis procedure, 1/8 patients presented a transitory degree 1 increase of transaminasas and hypertryglyceridemia.

No autoimmune reaction, after the infusion of pertinent effector lymphocytes, was observed. It was measured systemically in peripheral blood according to the lymphocyte proliferation index carried out on the day of the i.v. infusion, 48 h, 1 week and 1 month later.

Treatment efficiency

Patients with low cervical injury (patient 5) and thoracic injury (patients 4 and 6) followed a sensory and motor recovery pattern that descended along the trunk and limbs, from proximal to distal endings. This evolution was easily assessable by the ASIA (American Spinal Injury Association) scale and its functional degrees (A–E).

Sensory recovery had three different stages: lack of skin sensibility, followed by dysesthesia episodes that, in many cases, evolved to pain until they finally disappeared and led

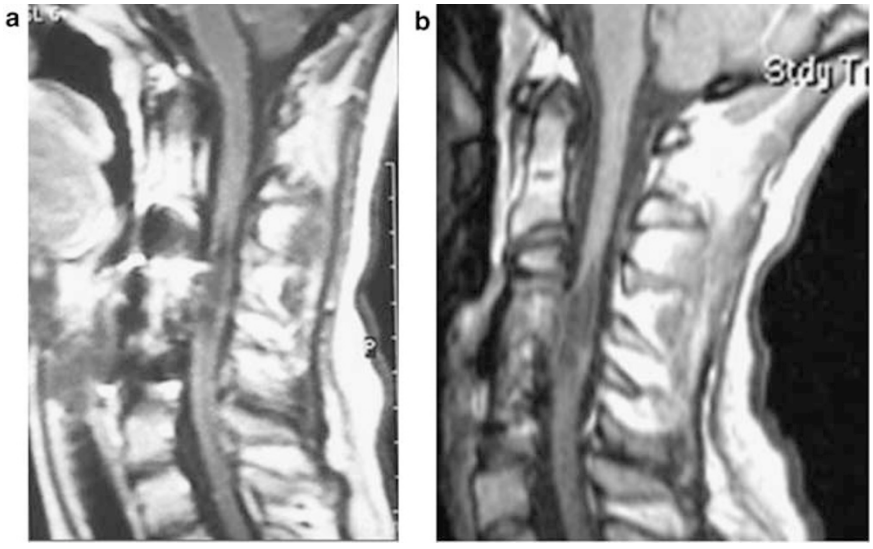

Figure 1 (**a**) MRI of patient 2 in March 2005. (**b**) MRI of patient 2 in June 2007. MRI, magnetic resonance imaging.

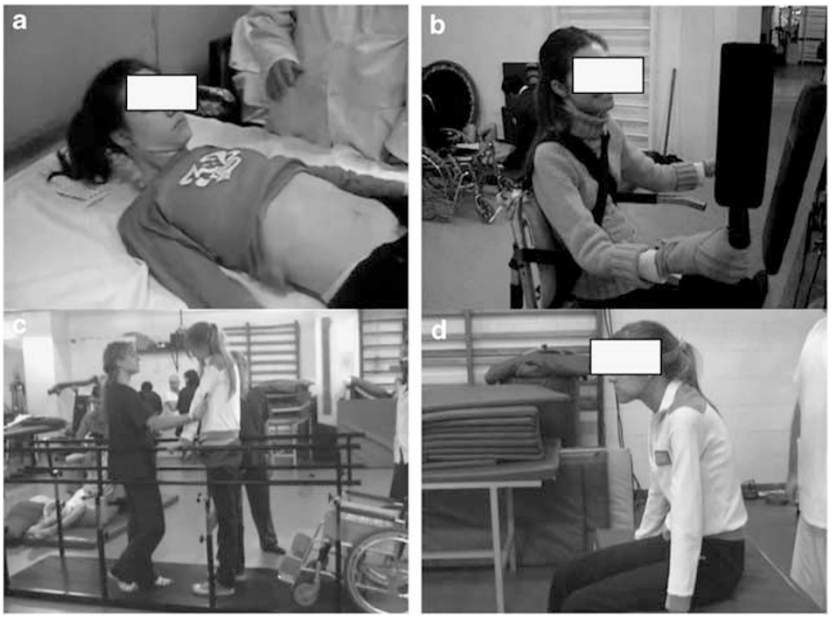

Figure 2 Pictures (**a** and **b**) illustrate that in March 2005, patient 2 could only move muscles that belonged to spinal cord roots C3–C4. To sit down or to move, any rehabilitation machine was needed. Pictures (**c** and **d**) illustrate that in May 2008, patient 2 could move muscles that belong to spinal cord roots S4–S5. She could sit down without any support and stand up with minimal support.

to a progressive increase from propioception to tactile discrimination.

Patients with chronic SCI suffered flaccid paralysis at both the injury level and below. Five of the eight patients experienced variable degrees of spasticity of the muscles below the lesion.

After BEN treatment, the functionality of spastic muscles was the first to recover. However, muscles with flaccid paralysis presented an increase in their tone at the first stage, followed by a second stage with predominant spasmodic contractions (producing occasional pain) and, finally, a gradual recovery of strength and muscle functionality. This process was cyclic and lasted for the whole follow-up period of patients treated and rehabilitated suitably (7/8), even in those who received the last BEN treatment 30 months earlier. When this rehabilitation program was interrupted or an unsuitable strategy was applied, the recovery process stopped, although the benefits achieved were not lost.

Patients with high cervical injury (patients 1–3, 7 and 8) achieved a similar sensory and motor recovery; however, the recovered metameric levels followed an atypical progression.

Although motor functions and sensibility of the spinal cord segments below the injury level followed patterns similar to that described earlier, the same functions of the injured metameric segments followed their own pattern that of slower recovery from proximal to distal areas.

In other words, although the patient was able to recover from head and trunk injury in a rapid progressive pattern, the upper limb motility and sensitivity showed slower rate of recovery. Such phenomena are taken as the difference between simply recovering cortico-spinal connections and the need to reconstruct the motor nucleus and sensory ganglia of the injured area, either by regeneration or by neuronal plasticity.

Objective quantification results of the Spinal Cord Functional recovery, carried out according to the scale transcribed in Table 1, are summarized in Table 2a and b.

Figure 1 summarizes the most significant cervical magnetic resonance imaging of patient 2. Figure 2 shows photographs of patient 2 taken at the beginning of treatment and 34 months after.

Discussion

This report suggests that a combined cellular therapy approach associated to a specific neurorehabilitation scheme may be feasible, safe and effective to promote neural restoration of chronic SCI patients. Further works are under process to certify these findings.

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