

Rehabilitation of spinal cord injury with autogenous stromal vascular fraction in dogs

Abstract

Background: Spinal cord injury (SCI) is an unexpected, traumatic event, its consequences often persist for the rest of life. The neurological deficit caused by spinal cord (SC) injury is permanent, and conservative treatment can not completely restore neurological functions in animals. Repeated autogenous adipose-derived stromal vascular fraction (SVF) has been used to deliver MSCs, growth factors such as brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 at the site of injury.

Methods: In the present study, six dogs presented with SCI, were routinely examined based on standard neurological examination protocol and subjected to the survey radiography. Treatment plans differed as per the presentation of the case. Stable and physically responsive patients were selected for repeated autogenous SVF therapy for rehabilitation.

Results: After conservative treatment in SCI, from the second week onwards, when patient supports or able to move affected area in relation to forequarter, the patients were administered repeated autogenous SVF therapy, which restored motor function in the form of intermittent weight-bearing on fetlock and/or posture change.

Significance: Spinal cord damage either partial or complete is always an ailment related to neurological deficit of the affected body parts. The repeated administration of autogenous stromal vascular fraction therapy in combination of physiotherapy significantly reverse, the effects of neurological deficit and progressively restore posture and weight bearing on the affected hind quarter in dogs.

Keywords: spinal cord injury, adipose-derived stromal vascular fraction and rehabilitation

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Abbreviations: SCI, spinal cord injury; SC, spinal cord; SVF, stromal vascular fraction; MSCs, mesenchymal stromal cells; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified Eagle's medium

Introduction

Spinal cord injury (SCI) is an unexpected, traumatic event, its consequences often persist for the rest of life. Recovery of motor, sensory and autonomic function loss after severe SCI is an unmet clinical demand due to lack of effective therapy.¹ Multidimensional pathophysiological cascades following SCI are responsible for the loss of function that eventually leads to the formation of unfavourable microenvironment which discourages regeneration.² The neurological deficit caused by spinal cord (SC) injury is permanent, and conservative treatment fails to completely restore neurological functions in animals. Due to unfavourable prognosis and permanent suffering of the severely affected animal, many owners consider euthanasia as a rational option for their pet (Sulla, et al., 2019). Primary mechanical damage due to SCI results in oxidative stress with local haemorrhage, oedema, and tissue necrosis. Subsequently, ischemia, anoxia, inflammation, apoptosis, and free radical production, occur over hours to days post SCI.³ Over the last two decades, numerous experimental studies have revealed various therapeutic measures capable of modulating the pathophysiological processes that cause cell death and the development of an irreversible SCI. Previous studies have shown that transplantation of mesenchymal stromal cells (MSCs) can reduce secondary damage and improve functional recovery after SCI.⁴ Autogenous stromal vascular fraction (SVF) has been used to deliver MSCs, growth factors such as brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 at the site of injury. However,

most transplanted cells died within the first few days post-transplant due to oxidative stress, hypoxia, and immune response.⁵ Keeping in view the present study was planned to increase the frequency of the SVF transplantation at the site of SCI for its rehabilitation.

Materials and methods

Spinal cord injury refers to trauma, that results in partial or complete loss of function (SCI - 2% out of which 60% were due to automobile accidents) symptoms ranges from ataxia to paralysis. Six (6) dogs having SCI presented at the Teaching Veterinary Clinical Complex, College of Veterinary Sciences and Animal Husbandry, Anjora, Durg (CG) during January to December 2019 were selected based on recovery from the acute trauma for repeated autogenous SVF therapy. All the animals were subjected to the standard line of the diagnostic protocol that includes patient history, physical neurological examination (included proprioceptive positioning reaction, visual placing reaction, nociception, withdrawal reflex, patellar reflex, perineal reflex, urinary control and followed by radiograph) (Bali, 2000). Initially animals with SCI were subjected to conservative treatment and rehabilitation therapy with SVF (intrathecally) on the day 14, 21, 28 and subsequent doses as per the recovery pattern. The details of patient data and clinical findings are presented in Table 1. Treatment protocols for the early reported cases of SCI included first-line care, involves the support of breathing and circulation, followed by an appropriate immobilization of the vertebral column to limit further damage of the spinal cord with the injection of corticosteroid methylprednisolone sodium succinate @ 5-10mg/kg bolus. Initial treatment with corticosteroid and analgesic followed by one to two weeks of treatment with multivitamin (mecobalamin, folic acid etc.) and gabapentin @ 5-7mg/kg in case of neurogenic pain was advised.

After conservative treatment, patients were subjected to autogenic SVF therapy with due consent of owners. Following pre-anaesthetic and anaesthetic plans were adopted as per the behaviour of the patient.

a) Premedicated with atropine sulphate @ 0.04mg/kg IM, followed by sedation with diazepam @ 0.5 mg/kg IV, plus butophenol @

0.2mg/kg IV and adipose tissue was collected from linea alba under local infiltration anaesthesia.

b) Premedicated with atropine sulphate @ 0.04 mg/kg IM, followed by propofol @ 5mg/kg IV bolus and adipose tissue was collected from linea alba.

Table 1 The detailed patient data and clinical findings of all patients

Case No	Age	Sex	Duration of illness	Breed	Physical status	Radiographic findings
1	2.5Y	M	2 W	ND	Paraplegia	L2-L3
2	5Y	F	1 W	Spitz	Paraplegia, back pain	T 12-13
3	1.5Y	M	3 D	Spitz	Paraplegia	L3
4	4Y	M	>2 W	ND	Monoplegia, sensory & motor deficit	S3+PF
5	6Y	F	>2 W	Lab	Paraplegia	L1-2
6	8Y	M	1 W	ND	sensory & motor deficit	Cd 3

In vitro procedures

Isolation and culture of canine adipose tissue-derived mesenchymal stromal vascular fraction: Canine adipose tissue-derived SVF was obtained as per the methods described earlier.⁶ Briefly, adipose tissue was aseptically collected under general anaesthesia from subcutaneous fat at linea-alba through undermining of skin in dogs. Written consent was obtained from the animal owner for all clinical procedures. The fat tissue was washed with phosphate-buffered saline (PBS) and digested with collagenase type I (1 mg/mL; Sigma-Aldrich, USA) for 45 min. at 37°C. The samples were then washed with PBS than incubated in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS; Gibco-BRL,USA) and centrifuged at 3000 rpm for 10 min. The pellet (the stromal vascular fraction) was re-suspended in 2 ml of RBC lysis buffer for 2 min. Then PBS was added to make the volume 10 ml, filtered through a 100 µm nylon mesh and centrifuged at 3000 rpm for 10 min. Supernatant was discarded and pellet was re-suspended in 10 ml PBS and filtered through the 100 µm nylon mesh and centrifuged at 3000 rpm for 10 min. Pellet re-suspended in 1 ml of PBS for in-vivo application.

In vivo application of the SVF

Intrathecal administration of the SVF: The site of injection was decided as per the radiographic findings and injection were

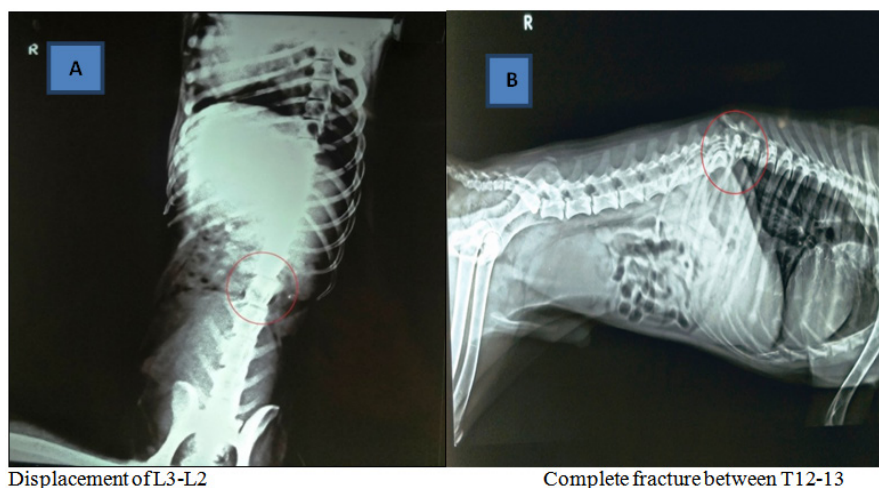
administered with 100-200 µl of SVF at three locations (middle of the injury site and at proximal and distal margins) to depths of 3 mm by using a 30-gauge needle.⁵

Results and discussion

Neurological deficit depends on the location, size and development of the SC lesion.⁷ Clinical symptomatology of SCI given by the arrangement of motor and sensitive tracts and spinal cord vessels includes tetra- or paraparesis/tetra- or paraplegia, urinary and faecal incontinence and sexual incompetence.⁸

Radiographic investigation

On survey radiography the patient were screened for the vertebral injuries as described in Table 1 and Figures 1A–1F, first case reflecting displacement of L3 in relation to L2, second case showed complete fracture between T12-13, third patient revealed compression in L3, fourth case had fracture of left pubic bone with compression of S3 and fifth and sixth case showed abnormality in both L1-2 and caudal 3 vertebrae. Plain radiography supports findings, formal interpretation for immobilizing the patient appropriately. The failure to adequately immobilize the spine owing to mechanism of injury diagnosis is a pitfall.⁹



Displacement of L3-L2

Complete fracture between T12-13

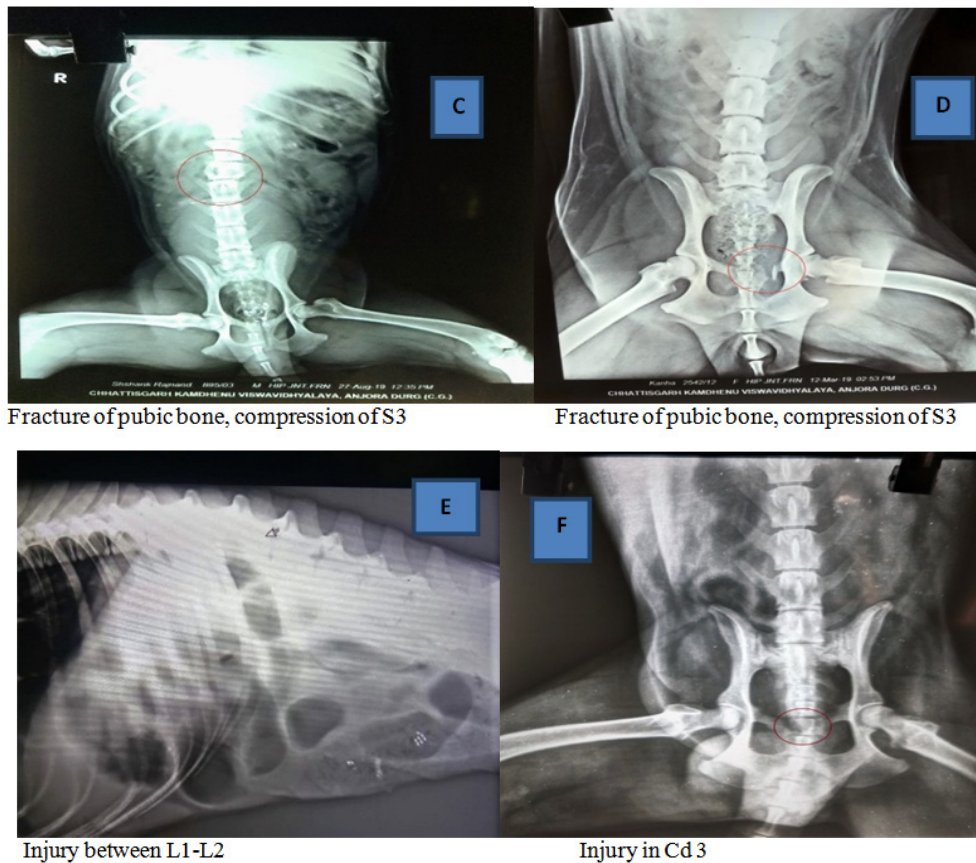


Figure 1A–1F Radiographic investigations.

After treatment of acute symptoms of SCI, from the second week onwards, when patient supports or able to move the affected area in relation to forequarter, the patients were given autogenous SVF, ability to intermittent weight-bearing on fetlock and posture change was considered as sign of recovery for SCI. After the second dose of SVF treatment, patients except for case2, started showing walking movement of forelimbs with incoordinated patterns which gradually improved with third SVF treatment and physiotherapy. The second case also started weight-bearing on a right hind limb and left hind limb did not respond to regenerative therapy. It has been reported that the

MSC and growth factors in adipose-derived SVF provide neuron-like cells, may help nerve fibre regeneration.¹⁰ Stem cells which normally occur in adult tissues maintain their viability during the whole life span by replacement of dead cells.¹¹ It is important to mention that trauma triggers SCI by different mechanisms and SVF provides MSCs, can expand, trans-differentiate to multipotent progenitors, replace damaged cells, produce growth factors and trophic mediators. Therefore, repeated administration of autogenous SVF has restored the lost motor function in canine with SCI. Table 2 and Figures 2A–2F.



Insetion of spinal needle



Figure 2A–2F Sketch diagram represent of weight bearing.

Table 2 The summary of detailed neurological examinations

Reflex	Grades	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Proprioceptive positioning reaction	3 (intact)	1	2	1	2	1	3
	2 (occasional)						
	1 (absent)						
Visual placing reaction	3 (intact)	1	1	1	1	1	2
	2 (occasional)						
	1 (absent)						
Nociception	3 (superficial pain intact)	1	2	1	2	1	3
	2 (absent superficial but deep pain present)						
	1 (absent deep pain)						

Table Continued...

Reflex	Grades	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Withdrawal reflex	2 (present)	1	2	2	1	2	2
	1 (absent)						
	LMN	LMN 2	LMN 2	UMN 2	3	UMN 2	3
Patellar reflex	1 (absent), 2 (occasional)						
	3 (normal)						
	UMN						
	2 (exaggerated), 1 (exaggerated with clonus)						
Perineal reflex	3 (intact)	2	2	3	2	1	2
	2 (occasional)						
	1 (absent)						
Urinary control	3 (intact)	1	1	1	2	1	3
	2 (occasional)						
	1 (absent)						

Conclusion

Spinal cord injury (SCI) a traumatic events in dog is clinically characterized by ataxia to paralysis. In the present study, SCI in dogs presented to TVCC, was diagnosed on the basis of physical, neurological and radiographic examination. Initial conservative treatment followed by repeated autogenous SVF therapy resulted restoration of lost motor functions through repairing of injured spinal cord in canine leading to rehabilitation.

Acknowledgments

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Conflicts of interest

The author states there are no conflicts of interest.

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