

Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial

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Olfactory ensheathing cells show promise in preclinical animal models as a cell transplantation therapy for repair of the injured spinal cord. This is a report of a clinical trial of autologous transplantation of olfactory ensheathing cells into the spinal cord in six patients with complete, thoracic paraplegia. We previously reported on the methods of surgery and transplantation and the safety aspects of the trial 1 year after transplantation. Here we address the overall design of the trial and the safety of the procedure, assessed during a period of 3 years following the transplantation surgery. All patients were assessed at entry into the trial and regularly during the period of the trial. Clinical assessments included medical, psychosocial, radiological and neurological, as well as specialized tests of neurological and functional deficits (standard American Spinal Injury Association and Functional Independence Measure assessments). Quantitative test included neurophysiological tests of sensory and motor function below the level of injury. The trial was a Phase I/IIa design whose main aim was to test the feasibility and safety of transplantation of autologous olfactory ensheathing cells into the injured spinal cord in human paraplegia. The design included a control group who did not receive surgery, otherwise closely matched to the transplant recipient group. This group acted as a control for the assessors, who were blind to the treatment status of the patients. The control group also provided the opportunity for preliminary assessment of the efficacy of the transplantation. There were no adverse findings 3 years after autologous transplantation of olfactory ensheathing cells into spinal cords injured at least 2 years prior to transplantation. The magnetic resonance images (MRIs) at 3 years showed no change from preoperative MRIs or intervening MRIs at 1 and 2 years, with no evidence of any tumour of introduced cells and no development of post-traumatic syringomyelia or other adverse radiological findings. There were no significant functional changes in any patients and no neuropathic pain. In one transplant recipient, there was an improvement over 3 segments in light touch and pin prick sensitivity bilaterally, anteriorly and posteriorly. We conclude that transplantation of autologous olfactory ensheathing cells into the injured spinal cord is feasible and is safe up to 3 years of post-implantation, however, this conclusion should be considered preliminary because of the small number of trial patients.

Keywords: human; transplantation; spinal cord injury; paraplegia

Abbreviations: ASIA = American Spinal Injury Association Impairment Scale; COVS = Clinical Outcome Variables Scale; FDI = first dorsal interosseous; FIM = Functional Independence Measure; IADL = Instrumental Activities of Daily Living; MRI = Magnetic Resonance Imaging; SSEP = somatosensory evoked potentials; TMS = transcranial magnetic stimulation; ZPP = zone of partial preservation

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Introduction

Olfactory ensheathing cells are specialized glia that surround the olfactory nerve fascicles as they project from the sensory epithelium to the olfactory bulb (Doucette, 1990). They have generated considerable interest as candidates for cell transplantation therapies for repair of spinal cord injury after numerous preclinical studies (Raisman, 2001; Mackay-Sim, 2005; Richter and Roskams, 2007). They are accessible in human via biopsy through the external naris (Feron *et al.*, 1998, 2005; Bianco *et al.*, 2004).

In 2002, we initiated a single-blinded, controlled trial to establish the safety and feasibility of intraspinal transplantation of autologous olfactory ensheathing cells in human spinal cord injury. An initial report described the surgical procedure and safety outcome of the trial after 1 year (Feron *et al.*, 2005). This report describes in detail the design of the trial and describes the results of clinical, psychosocial, neurological and neurophysiological assessments that were made every 3/6 months over the 3 years following cell transplantation.

In considering the design of a Phase I/IIa trial of a cell therapy in spinal cord injury, several aspects were paramount. Complete (American Spinal Injury Association Impairment Scale Grade A: ASIA A), thoracic level of injury was chosen to minimize the risk of loss of useful function above the site of injury. Choice of patients who had completed at least 6 months post injury would provide a homogeneous group with stable neurological and functional baselines from which to assess changes due to treatment. Psychosocial health and stability was considered important to counter potentially unrealistic expectations of clinical trial outcomes. It would also increase the likelihood of retaining the patients for the long follow-up period of the trial. Long-term follow-up was considered important for assessing risks associated with the development of cysts, syringomyelia or transplanted cell over-growth, or the development of neuropathic pain. Repeated assessment raised important issues of assessor variability and bias. Variability was controlled by using the same assessors throughout. Bias was controlled by having a control group of patients, with the assessors blinded to treatment group. The presence of a control group then raised the possibility of obtaining preliminary data on the therapeutic effect of cell transplantation, although this was not the primary aim of this trial.

Methods

Overview

Patients were recruited after the review of medical records and personal or telephone interviews. From over 600 such interviews, 12 persons were selected who initially fitted the inclusion criteria. These people took part in the intensive selection process in which they were interviewed and educated about the nature of the trial including aims, risks and design. These people were assessed medically and radiologically and undertook the initial 2 h

psychosocial assessment by psychiatrist and social worker. This assessment process selected six patients for the trial who agreed to take part as transplant recipients or as non-operated controls after being thoroughly informed of the trial and its attendant risks. The transplant recipients were counselled to expect no clinical benefit and they accepted that risk for altruistic reasons. Strict criteria were applied in order to select patients who were as homogeneous as possible within and between groups. Recruitment took place over a 2-year period so that the trial took 5 years to complete.

After recruitment, the patients were assessed to establish baseline measures. All transplant patients then received a nasal biopsy from which olfactory ensheathing cells were grown for autologous transplantation into the injured thoracic spinal cord of the transplant recipient group. Regular assessments then followed for 3 years. Patients were not instructed to follow any particular exercise regime and all undertook their usual daily activities. Throughout the trial, at each assessment from the initial selection through to completion, a rehabilitation physician with extensive experience in management of people with spinal cord injury, who was not blind to group status, acted as reviewing physician. The reviewing physician conducted an independent neurological examination of each patient at each assessment session. He examined each patient to monitor their health with the responsibility to immediately notify the trial manager and ethics committees of any adverse events, with the power to stop the trial.

The study was designed to maximize statistical power through the selection of homogeneous patients groups, a within subjects comparison (i.e. before and after), a non-transplanted control group, a single blind assessment regime, the same assessors throughout and a rigorous and long-term follow-up.

The trial was approved by the ethics committees of the Princess Alexandra Hospital, Griffith University and Queensland University of Technology, according to guidelines of the National Health and Medical Research Council of Australia. The trial was approved by the Therapeutic Goods Administration of Australia and accordingly monitored by the responsible ethics committees.

Selection criteria

The primary inclusion criterion was a traumatic injury of the thoracic spinal cord (T4–T10) occurring 6 months to 3 years prior to enrolment in the trial, resulting in a sustained and complete loss of sensory and motor function below the injury (ASIA Category A, 'ASIA A' classification). Exclusion criteria included: substance abuse, spinal vertebral instability, major concurrent medical illness (e.g. carcinoma, auto-immune disease, diabetes mellitus) and ASIA Impairment Scale category other than A. Syringomyelia was also an exclusion criterion but an exemption was made for a control patient with a stable syrinx. Patients with major and current psychiatric illness, who had significant traumatic brain injury associated with the spinal cord injury or who were otherwise considered unable to provide fully informed consent were also excluded. Additional selection was based on a 1-hour interview and psychosocial assessment performed by a social worker and psychiatrist (Fronck, 2004). The initial interview included an assessment of understanding of research project and risk factors, evidence of patient's ability to cope with a negative/positive result, assessment of significant other/family's attitude towards research and possible negative result and other issues raised during the interview. The purpose of the interview was to select patients with a stable social and family background,

who were committed to the trial, and who accepted the low probability of a change in function as a result of the trial, as well as the remote possibility that the implant procedure may cause deterioration in neurological function. This selection was considered necessary to reduce 'false hope' of recovery and to provide the highest probability of compliance with the demands of the trial over the 3-year assessment period.

Transplantation procedures

The nasal biopsy, olfactory ensheathing cell culture and surgical procedures are fully described elsewhere (Feron *et al.*, 2005). In brief, biopsies were taken from the olfactory mucosa under general anaesthesia and olfactory ensheathing cells were cultured from these biopsies for 4–10 weeks. After appropriate tests for cell sterility, they were transplanted into the injured spinal cord. A laminectomy was performed and, following a durotomy, cells were injected into the spinal cord at multiple sites throughout the damaged cord and into the proximal and distal ends of the intact cord. Transplantation and surgery were performed under microscopic control and using a purpose-designed injector device (Feron *et al.*, 2005).

Initial and repeated assessments

All assessments were performed with the assessors blinded to the group status of the patients. Before the assessments, the surgical scar of transplant recipients was covered with a bandage and a similar bandage was applied to the controls. Patients progressed through the trial in pairs, each assessed in the same sessions. Variability was reduced by the use of the same assessors throughout the study, obviating any inter-observer variability. Blinded assessors did not discuss the trial results with each other and the results of their assessments were not collated until the end of the trial. No results were revealed to patients or other trial team members during the course of the trial.

Pre-operatively, the transplant recipients were assessed for haematology, blood chemistry and urine microbiology and screening for HIV and Hepatitis B and C status. They also received chest and thoracic spine X-rays. They were examined by the otorhinolaryngologist before olfactory tissue biopsy (Feron *et al.*, 1998). All patients had their sense of smell tested on admission to the trial and 3 months later using a quantitative olfactory test (Mackay-Sim *et al.*, 2004).

Magnetic resonance imaging (MRI) undertaken on a 1.5 Tesla instrument by the Radiology Department, Princess Alexandra Hospital. MRI (T_1 -weighted with gadolinium and T_2 -weighted) was performed after selection for inclusion into the trial and at 6 months, 1, 2 and 3 years.

The health of the patients was monitored by the reviewing spinal injuries physician and a clinical nurse. The clinical nurse took regular physical observations (blood pressure, temperature, pulse respiration rate). The spinal injuries physician undertook a physical examination, assessed anterior ASIA sensory level of impairment, skin and bowel and bladder function. Health assessments were made every 3 months.

Mental health was assessed by a psychiatrist and social worker as part of the initial selection process and at each assessment point throughout the trial (Fronek, 2004). The psychiatrist made a clinical assessment of mental health status. The social worker took a social history and history of injury that included: the rehabilitation process, the adjustment to disability, current

social/legal/financial situation, current care needs and services, pain and alcohol/drug use. Psychosocial assessments were made at 1 week, 1 month, 3 months, 6 months, 12 months and 3 years.

Clinical Outcome Variables Scale (COVS) assessment was undertaken by an experienced spinal injuries physiotherapist (Campbell and Kendall, 2003). COVS was performed by each patient using standard instructions. If the patient felt unable to perform a skill, a score of 1 was recorded. A video was taken of each test completed by the patient. A second examiner was employed to validate the scoring. No patient was able to perform any of the ambulation items hence all these items were scored as 1. The physiotherapist also performed: respiratory function tests (spirometry) in both supine and sitting positions; an assessment of muscle tone and spasm using the Modified Ashworth Scale; a skin examination and recording of bowel and bladder self-reports; and ASIA classification of neurological function. Pain was assessed via interview using an in-house multidisciplinary pain-assessment tool that includes asking the patient to identify painful areas on the body identified on a body chart, to describe the pain using standard descriptors (burning, throbbing, sharp etc), and to identify temporal aspects of pain. Patients rated pain 'now', 'at its worst' and 'average level' and rated severity on a 10-point numerical rating scale as recommended (Bryce *et al.*, 2007).

An experienced spinal injuries occupational therapist undertook the Functional Independence Measure (FIM), a battery of hand function assessments, and Lawton's Instrumental Activities of Daily Living (IADL) (Lawton and Brody, 1969). The FIM and IADL were completed via interview/report rather than direct observation of skills. These assessments were made every 3 months.

A neurologist undertook an examination, independently of ASIA examination, that included history, pin prick, motor assessment and assessment of light touch and pin prick impairment level measured from the sternal notch anteriorly and the spinous process posteriorly. Another neurologist assessed somatosensory evoked potentials (SSEP) evoked in the sensory cortex after electrical stimulation of the sensory nerves in the legs. SSEP was the only assessment that was not performed by the same person throughout the study. Three assessors made these measurements. Neurological assessments were made every 3 months.

A neurophysiologist performed transcranial magnetic stimulation (TMS) of the motor cortex to evoke activity in the lower limbs bilaterally (tibial anterior, vastus lateralis) using both figure of eight and circular coils. The upper limbs served as positive controls by evoking movement in the first dorsal interosseous (FDI) muscles, stimulated with a figure of eight coil. Stimulation intensity of the FDI was set at 10% greater than the resting threshold for activation. Stimulation of the lower limb muscles was performed at the same intensity as for the FDI muscles and also at 90% and 100% of stimulator output. TMS assessments were made every 6 months.

Results

Patients

Patients were males, aged 18–55 years, with the period since their injury ranging from 18–32 months and were neurologically stable (Table 1). All had ASIA A spinal cord injury between T4 and T7 of traumatic aetiology with no evidence of ongoing recovery of spinal cord function.

Table 1 ASIA Assessment: level of injury and zones of partial preservation at baseline and 36 months

Patient ^a	Sex	Age (years)		Baseline ^b		36 months ^b	
				R	L	R	L
T1	M	46	Level	T7	T7	T7	T7
			ZPP	T9	T8	T9	T9
T2	M	30	Level	T4	T4	T4	T4
			ZPP	T5	T6	T5	T5
T3	M	23	Level	T3	T3	T3	T3
			ZPP	T6	T6	T5	T5
C1	M	18	Level	T3	T3	T3	T3
			ZPP	0	0	0	0
C2	M	55	Level	T2	T2	T3	T3
			ZPP	T4	T6	T4	T4
C3	M	36	Level	T4	T4	T3	T3
			ZPP	T5	T5	T4	T6

^aT 1–3: transplant recipients; C1–3: control patients, ^bR: right side; L: left side

Post-surgical recovery

Post-surgical recovery was unremarkable and there were no adverse findings at 1 year, as described previously (Feron *et al.*, 2005). Olfactory function was not affected by nasal biopsy, tested before biopsy and three months later with all patients scoring in the normal range on this test (Mackay-Sim *et al.*, 2004; Hummel *et al.*, 2007).

Independent medical conditions during the trial

Transplant recipient 1 had a pre-existing bladder calculus treated (cystoscopy and litholapaxy) 4 months after cell transplantation. Transplant recipient 3 was treated for epididymo-orchitis 15 months after cell transplantation. Control patient 3 was treated for pneumonia 3 months after enrolling in trial.

Radiology

The radiological findings in the transplant recipients were unchanged from the findings at 1 year post-transplantation (Feron *et al.*, 2005). As noted previously, only an extensive increase in spinal cord volume would be detectable with the 1.5T MR imaging available, and none was seen at 1, 2 or 3 years post-transplantation. There were no adverse effects of transplantation visible in any of the MRIs of the three transplant recipients at any time (Fig. 1). The control patients showed no change in MRI from baseline at entry into the trial. There were no changes in the volume of the cystic area at the injury site. In any patient, there was neither syringomyelia nor pseudomeningocele. No new masses were visible in any of the MRI images at the injection site or anywhere else in the neuraxis. The pre-operative sagittal alignment of the spine was maintained with no new external compression of the spinal cord.

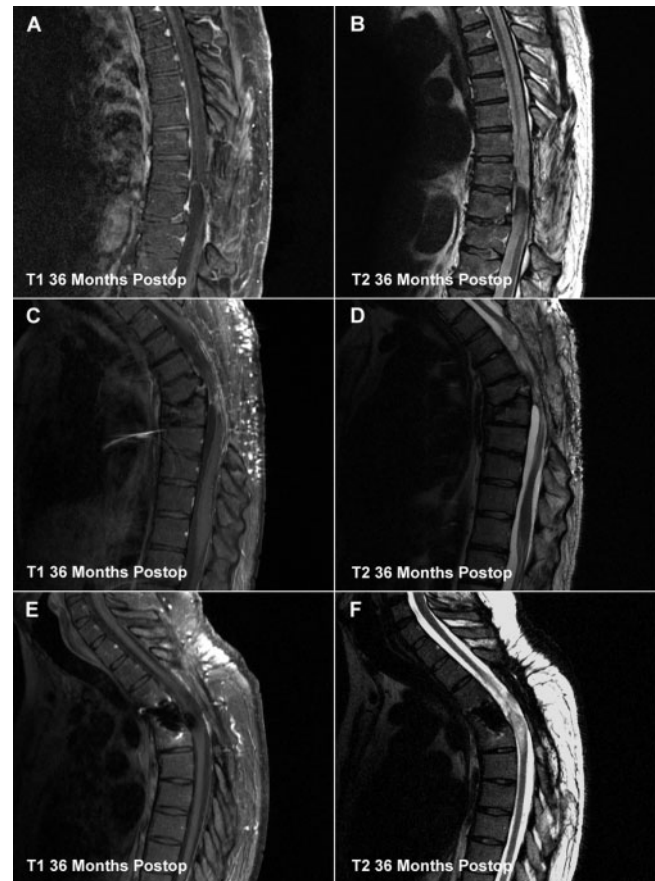


Fig. 1 Sagittal MR imaging of patients at 36 months after olfactory ensheathing cell transplants. Patients 1, 2 and 3 are shown as pairs (A and B, C and D, E and F, respectively). On the left are T₁-weighted images with gadolinium contrast. On the right are T₂-weighted images. Patient 3 has two cylindrical metal cages in the vertebral spaces, placed for treatment of the original vertebral column trauma, seen as white artifacts prominent in the T₁-weighted image.

Clinical assessment of transplant recipients at 3 years post-transplantation

Clinical and neurological findings at 3 years post-transplantation were unremarkable. There was no deterioration in neurological or functional level in any of the patients. There was no deterioration in sensory or motor function. There was no worsening of respiratory function. None of the patients experienced any new or additional neuropathic pain. There was no worsening in the severity of spasticity in any patients. Careful monitoring indicated that there was no worsening of the conditions over the period of the trial.

Psychosocial assessments

There was no deterioration in psychosocial status and all patients coped well. None of the patients had required any additional or unplanned counselling regarding the trial. There was no difference in psychosocial status between the transplant recipients and the controls.

Table 2 COVS: mean group differences between baseline and 36 month tests

COVS: maximum score 7 per item	Transplant		Control	
	Mean	SD	Mean	SD
1. Roll right/left side in bed	0.3	0.58	1.0	1.73
2. Get to a sitting position from supine lying in bed	0.3	0.58	0.0	0.00
3. Sitting balance	0.7	0.58	0.0	0.00
4a. Horizontal transfer	0.3	0.58	0.3	0.58
4b. Vertical transfer	-0.3	2.08	0.7	0.58
5. Performance of ambulation	0.0	0.00	0.0	0.00
6. Performance of ambulation with aids	0.0	0.00	0.0	0.00
7. Performance of ambulation—endurance	0.0	0.00	0.0	0.00
8. Performance of ambulation—velocity	0.0	0.00	0.0	0.00
9. Performance of wheelchair mobility	0.0	0.00	-0.3	0.58
10. Lt/Rt arm function	0.0	0.00	0.0	0.00

COVS

There were small variations in the COVS scores of patients. Table 2 shows the mean differences between baseline scores and scores at 36 months. There was no improvement in any of the subscales in either transplant recipients or controls.

Pain assessments

Most of the pain reported in the study was musculoskeletal pain or surgical pain that resolved normally. One transplant recipient had some neuropathic pain prior to enrolling in the trial. There was no ongoing neuropathic pain associated with the trial, with similar presentations by patients in both groups.

Respiratory function tests

Respiratory function tests were included to monitor respiratory status and to assist in detecting changes in intercostal and abdominal muscle function. Respiratory function was restricted as expected in patients with these levels of spinal cord injury. There were no significant changes in respiratory function over the course of the trial and no effect of transplantation on respiratory function as assessed by sitting and supine FEV1, FVC and FEV1/FVC ratio.

Functional assessments

There were no significant changes over the trial period in any of the measures of functional activity in daily life: FIM (motor), FIM (cognition) and IADL (Table 3). There was some variability noted in FIM locomotion scores where individuals were required to rate the amount of assistance they would require to go up and down a flight of 14 stairs. The variability depended on how the person was feeling on the day as to whether they would require one or two people to help. Some of the variability on the IADL scale could be attributed to changing functional skills irrespective of the

Table 3 Functional testing: mean group differences between baseline and 36 month tests

	Transplant		Control	
	Mean	SD	Mean	SD
FIMS motor (max. score: 91)	1.00	2.65	0.00	0.37
FIMS cognition (max. score: 35)	0.00	0.00	0.00	0.00
Lawton's IADL (max. score: 16)	0.00	0.00	0.67	0.00
Hand function grip L (kg force)	-3.57	4.70	-0.05	4.44
Hand function grip R (kg force)	-0.77	5.55	-0.13	2.81
Hand function Palmar pinch L (kg force)	-0.30	2.11	-1.49	1.07
Hand function Palmar pinch R (kg force)	0.20	2.26	-1.50	0.78
Hand function 3-point pinch L (kg force)	-0.40	2.29	-0.30	1.22
Hand function 3-point pinch R (kg force)	-0.40	2.91	-0.50	1.47
Hand function Lateral pinch L (kg force)	3.50	2.91	-1.03	1.29
Hand function Lateral pinch R (kg force)	3.77	3.09	0.00	1.40
9 Hole peg test L (sec)	-1.67	2.15	-2.55	2.06
9 Hole peg test R (sec)	0.18	2.22	-2.86	0.78
Preston grooved pegboard L (sec)	-1.44	3.79	-12.81	2.56
Preston grooved pegboard R (sec)	-4.75	0.99	-5.75	3.76

study. There was no effect of treatment on hand function: hand grip (Jaymar dynamometer); pinch strength; coordination (Hole Peg test, Preston Grooved Pegboard test). There was no effect of treatment on any of these tests as demonstrated in Table 3, which shows mean group differences between baseline and 36 month assessments.

ASIA assessments

There was no change in ASIA grade in any of the patients. There were minor variations in the zone of partial preservation (ZPP) for patients in both the control and surgery groups evident at 3 years (Table 1), but there were no consistent group differences. The motor and sensory scales were considered separately. The scores for both upper and lower motor scales remained the same for every assessment during the 3 years of the trial. The scores were universally 50 and 0 for each patient, for each assessment. There were minor variations in the sensory scores for light touch and for pin prick during the 3-year trial (Fig. 2). These minor variations in scores were due to differences in reported sensitivities close to the level of injury.

Neurological and neurophysiological assessments

The light touch and pin prick assessments made by the neurologist indicated significant variability in the transplant group, compared to the control group. When transplant recipient 1 was tested at 1 month, light touch and pin prick sensitivity extended further caudally compared to the initial measurements, on left and right sides and on front and back skin surfaces. This increased sensitivity was maintained for the rest of the 36 months observation period (Fig. 3). The extension of sensitivity in this Patient 1 was also noted by the reviewing physician who noted an

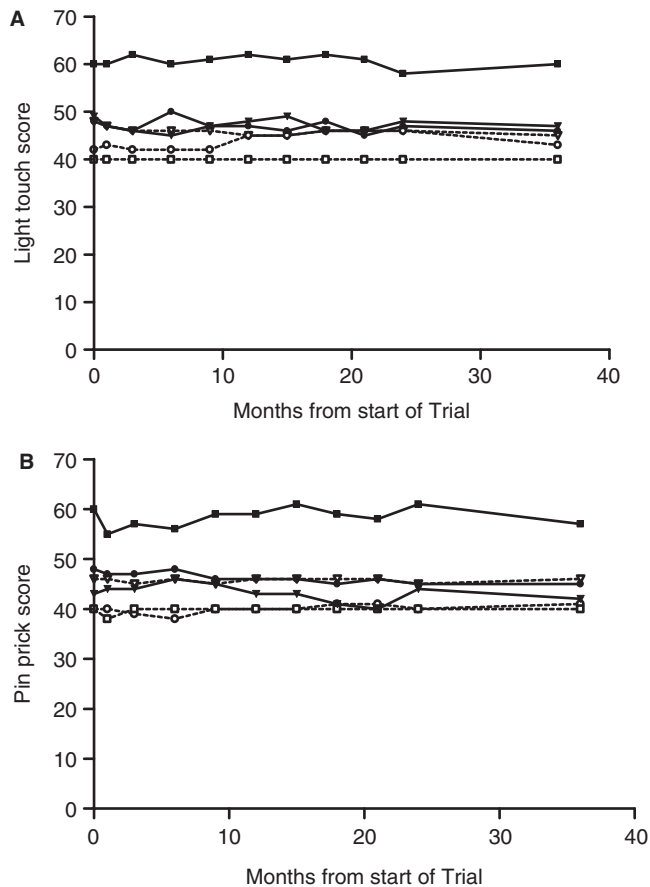


Fig. 2 ASIA sensory scores during the period of the trial. **(A)** Sensory scores for light touch in the transplant recipients (closed symbols, lines) and Controls (open symbols, dotted lines). **(B)** Pin prick scores for pin prick in the transplant recipients (closed symbols, lines) and controls (open symbols, dotted lines).

increase in light touch and pin prick sensitivity from T4 to T10. Light touch and pin prick sensitivity did not change in any of the other patients.

SSEPs were never evoked in any patient after electrical stimulation of lower sensory nerves. Control stimulations to upper limb sensory nerves reliably evoked SSEPs. TMS never activated lower limb muscles for either group even when stimulation was at 100% of coil intensity. The FDI muscles were activated in both of the upper limbs and the latency of activation remained constant for both experimental and control groups across all assessments.

Discussion

There were no adverse findings 3 years after autologous transplantation of olfactory ensheathing cells into the injured spinal cords of patients whose spinal cord injuries were incurred at least 2 years prior to transplantation. The MRIs at 3 years showed no change from pre-operative MRIs or intervening MRIs at 1 and 2 years, with no evidence of any tumour or cyst of introduced cells and no development of post-traumatic syringomyelia or other

adverse radiological findings. Similar stability in MRI findings was observed in both groups of patients, transplant recipients and controls. In most other measures, all patients showed very stable outcomes from pre-operative through to the final assessment at 3 years. The neurological assessment revealed small improvements in light touch and pin prick sensitivity in one transplant recipient anteriorly and posteriorly. This increased extent of sensation was observed at 1 month post-transplantation and remained for the rest of the trial. The reviewing physician noted the increase in sensitivity anteriorly in this patient but it was not noted by the physiotherapist undertaking ASIA sensory assessment. This suggests that the increased sensation was below threshold for clinical assessment.

Trial design

This study, initiated in 2001, was a controlled, single-blinded design which included a within-subjects comparison. In most respects, this design conforms to the recent recommendations of an international committee for the design of clinical trials in spinal cord injury (Fawcett *et al.*, 2007; Lammertse *et al.*, 2007; Steeves *et al.*, 2007; Tuszynski *et al.*, 2007).

Thoracic injuries were preferred in this first trial to minimize the risk of serious functional impairment should the procedure lead to damage above the level of injury (Tuszynski *et al.*, 2007). A period of 6 months was chosen in the present study to minimize the likelihood of spontaneous recovery among the patients (Fawcett *et al.*, 2007). This choice of complete, thoracic injuries at 6 months was demonstrated to have the most statistical power, requiring the fewest patients (Fawcett *et al.*, 2007). The planned size of the study was limited to a total of eight patients by the hospital and university ethics committees. The final size was limited by difficulties in recruitment due to the relatively low frequency of thoracic level spinal cord injury and the strict selection criteria.

The trial required repeated assessment of transplant recipients for 3 years. A non-operated control group provided a longitudinal comparison for the period of the trial. This control group assisted in assessing the probability of spontaneous recovery over the 3-year trial period. Spontaneous recovery causing a change from initial ASIA A grade may occur in up to 20% of persons when reassessed at 1 year after diagnosis (Fawcett *et al.*, 2007), although the majority of persons with thoracic spinal cord injury diagnosed ASIA A at 6 months will not change further by 1 year (Fawcett *et al.*, 2007). This was the minimum period for inclusion in the present study. Nonetheless, spontaneous recovery should not be disregarded because, at least after cervical injuries, 5.6% of persons diagnosed ASIA A at 1 year had changed the grade at 5 years (Fawcett *et al.*, 2007). Functional improvement in one individual was not recorded until after 5 years (McDonald *et al.*, 2002). Such statistics emphasize the

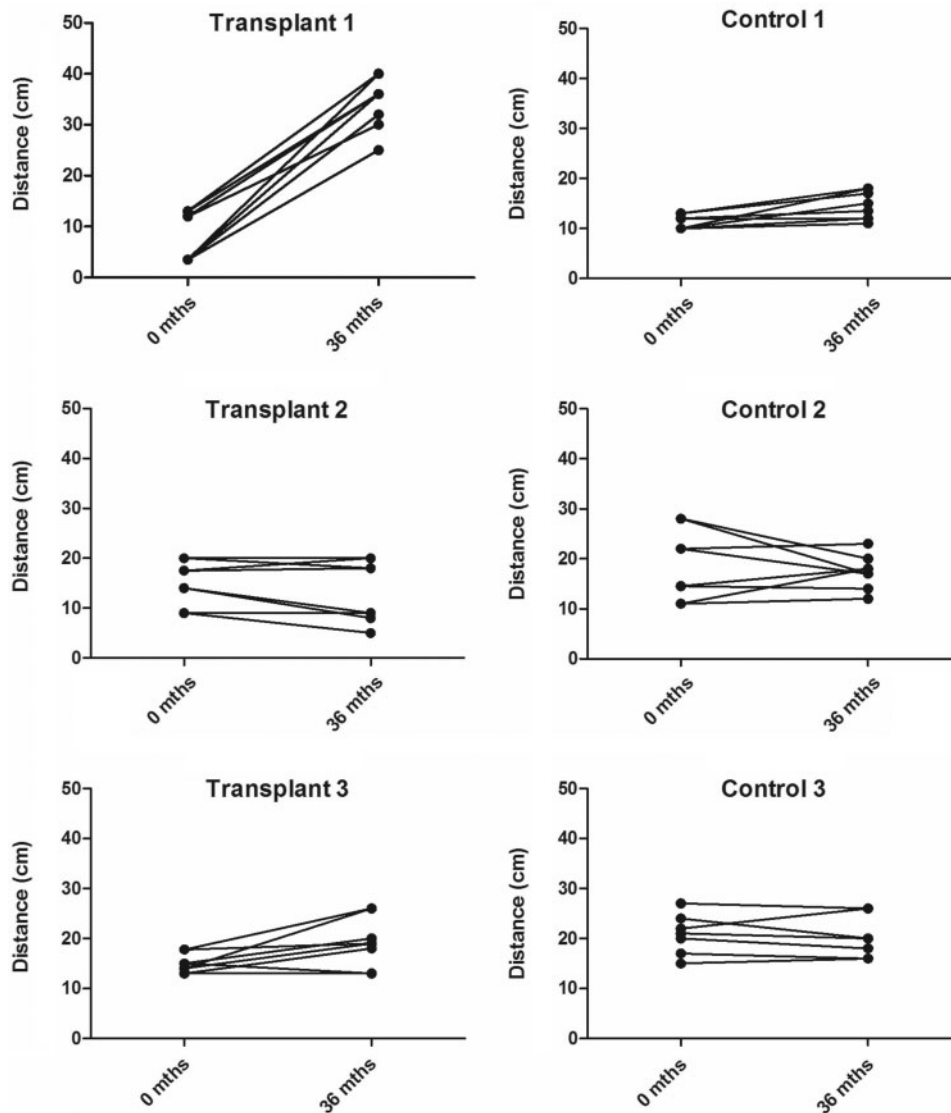


Fig. 3 Changes in light touch and pin prick sensitivity during the period the trial. The graph shows the differences in location of sensitivity to light touch and pin prick, anteriorly and posteriorly (8 measurements per patient) at baseline (0 months) and 3 years later (36 months). The locations were measured in centimetres from the sternal notch (anteriorly) and spinous process (posteriorly).

importance of a control comparison group. The statistics also emphasize the importance of the period since the injury in defining a stable pre-intervention baseline. Ideally, a sham-surgical control is preferred but in this trial, the risks associated with surgery raised significant ethical issues, highlighting the difficulties of achieving the 'best design' versus what is both ethically acceptable and practical. In addition, the surgical risks meant that the patients were not randomized to treatment and control groups. The acceptance of the surgical risk acted as a selection criterion, such that others agreed to act as controls, without wishing to be part of the transplant group. With informed consent being such an important part of the selection process, and with the numbers of patients being limited by type of injury, it then became impossible to randomize the selection process. The resultant trial design thus reduced without eliminating,

all the sources of bias inherent in clinical trials (Lammertse *et al.*, 2007).

Blinded assessment is considered essential to eliminate bias in clinical trials because of the qualitative nature of clinical examinations (Lammertse *et al.*, 2007). Assessor bias was reduced in the present study by using the same assessors throughout and by blinding them to patient treatment. For clinical examinations, two assessors made their assessments and reports independently and without consulting the other. Another possible source of bias may have been the necessary selection of highly motivated patients who may have under-reported negative effects such as pain, discomfort and psychological disappointment. The presence or impact of such bias is unknown but in this and future trials the inclusion of a similarly highly motivated control may help reduce any effect of such bias.

Although olfactory mucosa transplants were used in human trials (Lima *et al.*, 2006) and animal studies demonstrated the efficacy of whole olfactory lamina propria (Lu *et al.*, 2001, 2002), the present study used a purified population of olfactory ensheathing cells (Bianco *et al.*, 2004) in order to reduce variability among the transplants. Additionally, this was considered to reduce the probability of adverse outcomes such as overgrowth of cells. Olfactory mucosa, including lamina propria, contains many other cell types including endothelium, fibroblasts and glandular cells whose behaviour in the spinal cord are not known. Also present are olfactory stem cells (Murrell *et al.*, 2005), which are efficacious after transplantation into the injured spinal cord (Xiao *et al.*, 2005) but their long-term proliferation capacity is not defined. In contrast, olfactory ensheathing cells cease proliferating after transplantation into the spinal cord (Deng *et al.*, 2006).

Long-term follow-up was considered a necessary part of the design of any initial trial of the safety of olfactory ensheathing cell transplantation. Although this procedure was safe at 1 year (Feron *et al.*, 2005), 3 years was considered necessary to assess the possibility of the development of tumour, cyst or syrinx at the site of transplantation.

There is debate about the efficacy of exercise and rehabilitation in improving outcomes after spinal cord injury. Nonetheless, if neural circuits were rebuilt by an intervention then it is reasonable to expect a better functional outcome if the regenerating neuronal circuits are active, as demonstrated after regeneration of optic nerves in lizard (Beazley *et al.*, 2003) and motor nerves (Pavlov *et al.*, 2008) but not mixed nerve (Sinis *et al.*, 2008) in rat. Appropriate exercise, when combined with olfactory ensheathing cell transplantation significantly improved motor function, compared to cell transplantation alone in rat (Kubasak *et al.*, 2008). The design of this trial did not include exercise. Its primary goal was to test the safety of the transplantation procedure over a 3-year period. For this goal, patients were asked to report once in three months for assessment during their already busy lives. In the trial design, compliance for assessment of negative outcomes was considered more important than exploring the effect of exercise. In any case, the homes of the participants were located in city and in country areas, some hundreds of kilometres from the clinic, so application of a supervised exercise regime would have been impractical.

Finally, this study was designed with an emphasis on the psychosocial health of the patients, initially as part of the selection process and subsequently as part of the assessment of the outcomes of the trial. A stable psychosocial profile was considered important because of the highly emotive aspects of new experimental therapies in spinal cord injury and the potential for unrealistic expectations of some people with spinal cord injury. Fully informed patients were chosen with little expectation of recovery through this trial. This stability may have contributed to the retention of all

patients for the 3 year course of the trial, an essential requirement for already small group numbers.

Outcome measures

Recent reviews discuss the strengths and weaknesses of various outcome measures available and under development for spinal cord injury (Bryce *et al.*, 2007; Lam *et al.*, 2008; Steeves *et al.*, 2007). Of those used here, the ASIA scale measures neurological function and the FIM scale measures useful functional ability. Although these may be insensitive to small changes in physiology, which are of interest scientifically, both scales are well validated and used in clinics throughout the world. The ASIA scale is made up of sensory and motor components and these are useful measures of specific and smaller changes due to the experimental treatment. The additional neurological assessments in the present study indicate that more intense investigations of light touch and pin prick can reveal small, variable, sensory changes that are not obvious in a standard ASIA examination. The FIM tool is a valid and reliable instrument for functional independence of many patients, including those with spinal cord injury (Ottenbacher *et al.*, 1996). This standard clinical tool was chosen to monitor whether the cell transplantation procedure had an adverse effect on functional independence. After the start of our trial, a specialized instrument [Spinal Cord Independence Measure (SCIM) II] was introduced for assessing functional independence specifically after spinal cord injury (Catz *et al.*, 2001). The SCIM scale is still undergoing modification (Catz *et al.*, 2007) but future trials should consider using it instead of FIM because of its specificity for spinal cord injury which may make it sensitive to improvements in function as a result of the intervention as demonstrated by its ability to follow changes during the first year after complete injury (Wirth *et al.*, 2008). Future trials would benefit from validated measures of pain assessment that are now available (Bryce *et al.*, 2007). The COVS is a mobility scale that has construct validity for spinal cord rehabilitation when its subscales are recognized (Campbell and Kendall, 2003).

Future trials should consider including quantitative tests of sensory function, developed recently, which can identify small changes around the site of injury in complete thoracic injuries although these need further validation for general application (Savic *et al.*, 2006, 2007). Such quantitative measures may pick up small changes that will be important scientifically, and will direct future trials, but unless the changes are enough to alter clinical measures, such as ASIA scores, they will not have much impact on the patient. The present study included quantitative electrophysiological assessments (somatosensory evoked potentials, transcranial magnetic stimulation) for measurements of sensory and motor function in the lower limbs. Assessment of thoracic spinal cord function is noted as particularly problematic (Steeves *et al.*, 2007). In agreement with this, we initially

included intercostal electromyography which was discontinued because of variability around the level of injury. Recently, more fine grained and validated test tools and protocols have become available for assessing thoracic motor function (Ellaway *et al.*, 2007), which may be more appropriate for future trials when sub-clinical outcome measures are required.

MR imaging provided a qualitative, clinical assessment of the extent of injury and any changes during the course of the trial. These images were taken at a clinical radiology department with an instrument with 1.5T field-strength. Clinical instruments with a field-strength of 3T are now commonly available and these would provide better resolution in future trials, perhaps combined with diffusion tensor imaging (Ellingson *et al.*, 2008), although such quantitative analysis is yet to be validated in spinal cord injury. For the moment MRI remains a qualitative measure (Steeves *et al.*, 2007). Higher resolution MRI may allow detection of transplanted cells. Olfactory ensheathing cells labelled with iron oxide paramagnetic particles are detectable with MRI and survive for at least 2 months after transplantation into the spinal cord in rat (Dunning *et al.*, 2004; Lee *et al.*, 2004). A limitation is that it is not possible to distinguish the MRI signal of living transplanted cells from dead cells or macrophage or microglia that may phagocytose the label during an inflammatory process.

Clinical transplantation of olfactory tissues

A recent report described a clinical study of autologous transplantation of olfactory mucosa in human spinal cord injury (Lima *et al.*, 2006). In seven patients, pieces of olfactory mucosa were placed into the spinal cord after removal of the scar tissue, at 6 months to 6.5 years post-injury. Patients were assessed pre-operatively and followed for 18 months post-operatively. The transplant procedure was considered safe, with minimal adverse outcomes: temporary pain for 2–3 months in the trunk and lower limbs in two patients and the loss of sensory component of the ASIA score in one patient. Six patients gained sensory and/or motor function whereas one patient lost sensory function but gained motor function as measured on components of the ASIA scale. Two patients changed their ASIA grade from A to C. Some patients improved their bladder and bowel control. It was concluded that this transplantation procedure was ‘fairly safe’ and ‘may promote functional recovery’ (Lima *et al.*, 2006). Another report describes transplantation of fetal ‘olfactory ensheathing cells’ into the injured spinal cord in 300 patients with complete spinal cord injury (ASIA grade A) and incomplete spinal cord injury (ASIA grade B–D) (Huang *et al.*, 2006). Although the cells were derived from the olfactory bulbs of 12- to 16-week-old human fetuses, their identity as ‘olfactory ensheathing cells’ was not demonstrated and doubtful (Dobkin *et al.*, 2006). These allograft transplants were made without immune suppression and the patients

were followed for 2–8 weeks after transplantation. There was apparently ‘no fever, spinal cord infection, functional deterioration or mortality’ associated with the procedure (Huang *et al.*, 2006). Remarkably, 117 patients were reported to improve their ASIA scores 2–8 weeks after the procedure, most of whom began to improve within 2–3 days of the procedure (Huang *et al.*, 2006). An independent examination of seven patients who underwent the same procedure concluded that ‘patients have encountered serious medical complications and no lasting increase in sensorimotor function or functional ability’ (Dobkin *et al.*, 2006). Neither of these previous studies was a controlled clinical trial, raising questions about the validity of conclusions of improved function. Patient function was measured pre-operatively, providing a baseline with which to compare post-operative function, but there was no independent assessment of possible longitudinal changes, assessed with a control group, nor was the assessment blinded.

The current trial has raised no safety-related issues resulting from transplantation of autologous olfactory ensheathing cells into the injured human spinal cord, either at the time of surgery or at any time during the 3-year follow-up period. Despite the careful design of this trial, its findings must be considered preliminary because of the small numbers of patients involved. The finding that one transplant recipient had minor but measurable improvement in sensation below the original level of injury is noteworthy and encouraging for future trials. The optimal timing for olfactory ensheathing cell transplantation is unknown. Certainly delayed transplantations of olfactory ensheathing cells are efficacious in rat models (Lu *et al.*, 2002; Keyvan-Fouladi *et al.*, 2003; Plant *et al.*, 2003), but the maximum delay was only 1 month, rather than 2 years. It is possible that earlier transplantations may have a better chance of succeeding in humans.

The injured spinal cord is still changing in many patients during the first year (Marino *et al.*, 1999), and transplantation during this period may be more effective. Similarly, with time, the function of spinal neuronal circuits degrade caudal to the injury (Dietz and Muller, 2004) and earlier intervention and/or exercise to maintain those circuits (Dietz and Curt, 2006). Nevertheless, spinal neuronal circuits may still retain the ability to recover after a long time period, because 5.6% of patients who had neurologically complete injury at 1 year improved their ASIA grade at 5 years (Kirshblum *et al.*, 2004).

The primary aim of the present trial was to test safety, rather than efficacy. For the latter, such trials will require larger numbers in order to have appropriate power to overcome the increased variability due to spontaneous recovery (Fawcett *et al.*, 2007). Recruitment and selection of appropriate patients into larger trials will be a challenge, as our experience has demonstrated, and is likely to require national and/or international multi-centre collaborations.

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