Towards treating spinal cord injury in ‘patients’: one step at a time

For many years, there has been an expectation that developments in neurobiology and work on regeneration of the CNS will have early applications in the area of spinal cord injury. Now, a study by Granger et al. (2012) published in this issue of Brain reports a clinical trial in adult dogs with chronic spinal cord injury caused by an acute event, such as disk herniation. These injuries are common in veterinary practice, and studying therapeutics in this clinical population is sensible. The experimental therapeutic intervention was intraspinal transplantation of cultured autologous cells derived from the olfactory mucosa of the frontal sinus, whereas the control group received injections of cell culture medium only. The data analysis indicated a significant difference between the transplanted and control groups in forelimb–hindlimb coordination measured using kinematic analysis of locomotion on a treadmill during partial weight support. No difference in long tract functional recovery was detected between the groups using motor- and somatosensory-evoked potentials. This veterinary study has relevance to human clinical trial designs in several ways. For example, as the authors point out, the external validity is higher with this heterogeneous dog population than with a genetically homogeneous laboratory animal study. In addition, the cell therapy study had a relevant control group that was injected with cell culture medium and was blinded. Careful reading of the study discloses several interesting points for consideration.

It is not clear from the inclusion and exclusion criteria whether the enrolled dogs had undergone decompressive surgery at the time of their index paraplegia. Lack of surgical decompression might mitigate against recovery independent of a transplant effect. At study entry, there was a clinical examination, MRI, measurement of somatosensory-evoked potentials and motor-evoked potentials and urodynamic evaluation. The results of the MRI are not described. Motor-evoked potentials were recorded from one lower extremity muscle on each side, the cranial tibialis. It should be pointed out that the absence of signal does not mean there is no connectivity of the tested pathways, just that the signals were not found. The minimal number of functioning axons needed to elicit detectable somatosensory- or motor-evoked potentials is not known, and the neurons are affected by phenomena such as poor temporal summation in damaged pathways. It would have been beneficial to have included a quantified open field locomotor exam, as treadmill locomotion provides sustained afferent inputs that can elicit stepping. ASIA-A classification on the American Spinal Injury Association scale for spinal cord injury inputs that can elicit stepping. ASIA-A classification on the American Spinal Injury Association scale for spinal cord injury.

The enrolled dogs might be judged more like incomplete ASIA-B or -C human subjects. Kinematics simplifies the quantification of normal and abnormal motion. As the authors acknowledge, it is unlikely that a kinematic analysis could be accepted as the primary outcome measure in a human efficacy study. Dogs are a quadripedal species that requires forelimb–hindlimb coordination. Other animal spinal cord injury models also emphasize this behavioural end point (Basso et al., 1995), but its significance to human neurological function and recovery is not known. Olfactory ensheathing cells have been of interest for spinal cord repair for at least 15 years owing to their unique biology within the primary olfactory pathway. Peripheral olfactory sensory axons pass from the olfactory mucosa into the CNS at the olfactory bulb. Further, newly born olfactory sensory neurons can extend processes along this pathway throughout adult life. It was hypothesized that if olfactory ensheathing cells could have a similar function in an injury region, they might serve as an effective conduit for regenerating axons through the transitional zones observed between damaged and intact spinal cord tissue (Ramon-Cueto and Nieto-Sampedro, 1994). Persuasive data that olfactory ensheathing cells exhibit this phenomenon within the injured spinal cord are limited but have been reported in various specialized experiments, mainly using olfactory bulb nerve fibre layer-derived olfactory ensheathing cells (Li et al., 1998). Notwithstanding, a large number of experiments have tested purified cultured olfactory ensheathing cells, including a completed Phase 1 human clinical trial (Mackay-Sim et al., 2008). In other studies, biopsied olfactory mucosa was directly transplanted into the spinal cord (Lima et al., 2006, 2010). Concerns related to this strategy, as compared with injecting purified cultured cells, are that the olfactory mucosa is a mixed tissue containing numerous cells types and is highly variable among human individuals. The most assertive proponent of this therapy, Carlos Lima, who died earlier this year, published reports of human application in chronic spinal cord injury. The reported success was not replicated in an independent study (Chhabra et al., 2009). Nevertheless the weight of evidence supports some beneficial effects for transplanted olfactory ensheathing cells.

It is unlikely that regulatory approval for a human trial would be obtained without a reliable cell culture process generating more consistent proportions of olfactory glia versus fibroblasts than reported in this study. The Food and Drug Administration requires that the cell product manufacturing process for autologous cells leads to reproducible cell populations showing similar potency across different preparations. Heterogeneity of primary cell cultures has been a consistent issue reported in olfactory ensheathing cell studies from olfactory mucosa (Choi et al., 2008). Here, the authors selected for spindle-shaped cells (presumptive olfactory ensheathing cells) by differential trypsinization, dish agitation, serum reduction from 10 to 2.5% and use of the mitogens forskolin and heregulin, known to enhance the proliferation of...
Schwann cells and olfactory ensheathing cells. Cells were characterized by immunolabelling for p75 and fibronectin. At least 5 million cells of which, on average, one-half were p75-positive were generated over 3–5 weeks. It is not possible to determine from these methods whether the p75-positive cells included Schwann cells, which are also found in the olfactory mucosa of several species (Herrera, 2005). A total of 400 μl containing 5–6 million cells (~12,500 cells per μl) was injected. This is a substantial volume and the rate of injection is not stated. High injection rates can cause spinal cord tissue dissection (Guest et al., 2011). It is notable that the authors obtained more consistent cultures in their Phase 1 study when the primary tissue was from the olfactory bulb (Jeffery et al., 2005). On inspection of Supplementary Fig. 3, there appears to be a trend that the proportion of p75/fibronectin-positive cells increased in successive dogs, perhaps owing to improving techniques over the study duration. There was no correlation between p75 proportion of the transplanted cells and outcome. This finding is unexpected and raises the question as to whether the observed effect was due to the activity of the olfactory glial cells.

It is attractive to be able to perform percutaneous cell transplantation (Casas and Guest, 2004) as was used in this clinical trial. This technique allowed the use of culture medium controls and blinding by obviating a need for open surgery. However, there are risks to this method as compared with open surgery and direct spinal cord visualization. Three needles were placed by fluoroscopy to penetrate the spinal cord at the lesion site, and the adjacent rostral and caudal spinal levels. Subarachnoid penetration was verified by the presence of CSF return from the needles that were directed into the anterior spinal canal and then retracted into the mid-spinal cord parenchyma. This is quite a bold strategy because the risk factors associated with spinal cord perforation such as haemorrhage and physical compressive trauma cannot be visualized. In addition, deep placement to the level of the anterior spinal canal could allow the cell injectate to leak into the ventral spinal subarachnoid space and seed other locations in the neural axis. Further, the anterior spinal artery is a critical structure for blood supply to the spinal cord and might be damaged by this technique. This method might also cause iatrogenic cord injury because the spinal cord would be moving owing to ventilation and arterial pulsation, and the needle is rigidly fixed by the tissue it has passed through. With further imaging guidance developments, such a method might be applicable in humans, although in the context of incomplete spinal cord injury, its safety would require substantial evidence. The authors performed an analysis known as a Cohen’s d-value to quantify ‘effect size’. This analysis involves subtracting the end point control and treatment means and dividing by the variation. One curious aspect of the data is that the control animal’s forelimb–hindlimb coordination worsened over the study duration. Had this not occurred, it would have been unclear whether the group difference would be statistically significant and the effect size would have been reduced.

This team of academic veterinary surgeons has conducted a well-designed randomized blinded placebo controlled trial in a heterogeneous population of dogs with spinal cord injury. They observed an improvement in forelimb–hindlimb coordination, assessed using a treadmill body weight support kinematics paradigm. They did not observe major changes such as recovery of weight-supported stepping and useful locomotion. The authors propose that the improved coordination suggests that intraspinal circuits such as propriospinals were involved. Their work supports the premise that, in combination with other effective treatments, olfactory mucosal cell therapy may have a role in the treatment of human spinal cord injury.

James Guest1,2
1Department of Neurological Surgery, University of Miller School of Medicine, Miami, FL 33136, USA
2The Miami Project to Cure Paralysis, University of Miller School of Medicine, Miami, FL 33136, USA

Correspondence to: James Guest, MD, PhD, FACS
Department of Neurological Surgery and the Miami Project to Cure Paralysis
University of Miller School of Medicine
1095 Northwest 14th Terrace
Miami, FL 33136, USA
E-mail: jguest@med.miami.edu

doi:10.1093/brain/aws294

References