Understanding Cell Migration After Direct Transplantation into the Spinal Cord A Tool to Determine the Optimal Transplantation Volume

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Introduction

Cell therapies represent a promising alternative treatment for neurodegenerative diseases of the spinal cord, and traumatic spinal cord injury. Cell survival, migration, proliferation and differentiation are intrinsic factors that greatly influence the therapeutic potential of cell therapies. Other factors like local inflammatory and immune response also play an important role.

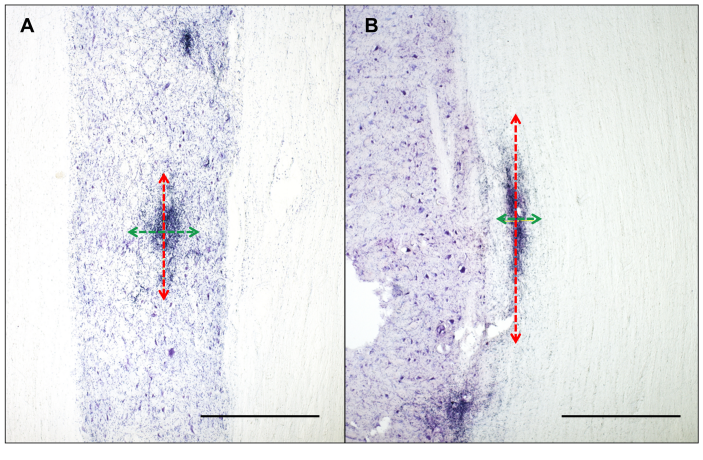
Main Ojective

This study analyzed the migration patterns of human fetal-derived neural precursors (hNPCs) transplanted to the spinal cord of healthy Gottingen minipigs.

Methods

Fifteen female minipigs divided into 3 groups received twenty bilateral 10, 25, and 50-microL intraparenchymal injections of hNPCs at a concentration of 10,000 cells/microL. Following 21 days, animals were euthanized, perfused, and spinal cords were harvested for immunohistochemistry.

Figure 1. Description of the method used to measure cell migration with Image J software.



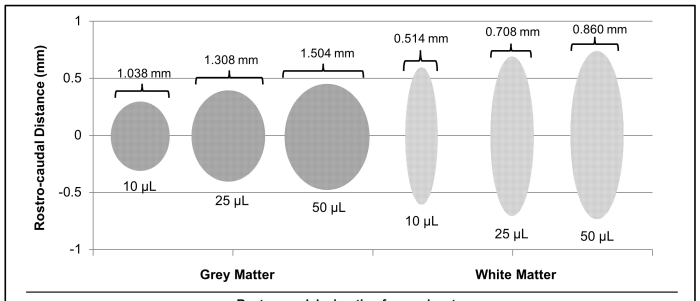
Migration was measured in the grey (A) and white (B) matter using a length probe placed through the graft epicenter from the furthest points where HuNu positive cells resided in the rostro-caudal (red arrows) and transverse (green arrows) planes. Scale bars=1mm.

Cell grafts (n=5 per group) in both white matter (WM) and grey matter (GM) were quantitatively assessed in the three-dimensional space using stereological volumetric calculations, and in the two-dimensional space using Image J software to estimate migration distance from the epicenter in the rostro-caudal and transversal planes.

Results

Cell grafts exhibited different migration patterns in each anatomical compartment, regardless of injection volume. Cell grafts found in the WM migrated more in the rostro-caudal plane, whereas cell grafts found in the GM migrated similarly in the rostro-caudal and transversal planes. The 50-microL grafts in the WM were significantly wider (p=0.02) when compared to the 10-microL grafts, but not significantly wider when compared to the 25-microL grafts. Additionally, the total volume of GM occupied by the 25-microL grafts was significantly larger (p=0.02) when compared to the 10-microL grafts, but not significantly smaller when compared to the 50-microL grafts.

Figure 2. Rostro-caudal and transverse cell migration in white versus grey matter.



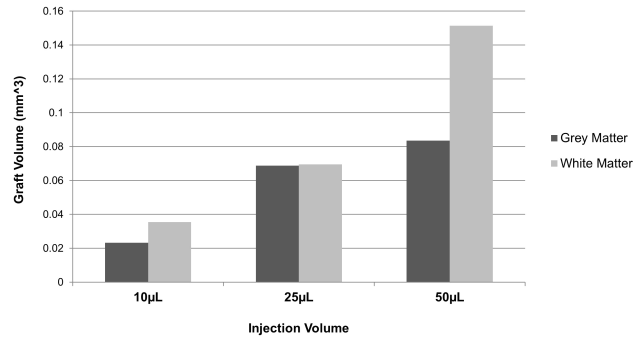
Rostro-caudal migration from epicenter				
	Injection Volume			
Injection Location	10 µL (n=5)	25 µL (n=5)	50 µL (n=5)	ANOVA
Grey Matter	0.619 ± 0.195 mm	0.818± 0.375 mm	0.946 ± 0.123 mm	0.16
White Matter	1.116 ± 0.412 mm	1.304 ± 0.161 mm	1.374 ± 0.243 mm	0.38
T-test	0.04*	0.03*	<0.01*	
Transverse migration from epicenter				
	Injection Volume			
Injection Location	10 µL (n=5)	25 µL (n=5)	50 µL (n=5)	ANOVA
Grey Matter	0.519 ± 0.182 mm	0.654 ± 0.333 mm	0.752 ± 0.244 mm	0.39
White Matter	0.257 ± 0.090 mm	0.345 ± 0.072 mm	0.430 ± 0.102 mm	0.03§
T-test	0.02*	0.08	0.03*	

§ Post-hoc t-test: 10 vs. 25 (0.3), 10 vs. 50 (0.02*), 25 vs. 50 (0.32); * Significance (α=0.05)

Data is expressed as mean +/- std. dev. (Aplha=0.05).

These results suggest that 25 microL is the optimal injection volume at a cell concentration of 10,000 cells/microL.

Figure 3. Estimated volume occupied by cell grafts using different injection volumes.



	Estimated Graft Volume			
	Injection Volume			
Injection Location	10 µL (n=5)	25 µL (n=5)	50 µL (n=5)	ANOVA
Grey Matter	0.023 ± 0.008 mm³	0.069 ± 0.003 mm³	0.084 ± 0.012 mm³	0.03§
White Matter	0.035 ± 0.010 mm³	0.070 ± 0.015 mm³	0.151 ± 0.091 mm³	0.01§§
T-test	0.07	0.96	0.14	

§ Post-hoc t-test: 10 vs. 25 (0.02*), 10 vs. 50 (<0.01*), 25 vs. 50 (0.57)
§§ Post-hoc t-test: 10 vs. 25 (0.59), 10 vs. 50 (0.01*), 25 vs. 50 (0.08)
* Significance (α=0.05)

Data is expressed as mean +/- std. dev. (Alpha=0.05).

Conclusions

Understanding the migration patterns and other dynamics of different cell lines will allow neurosurgeons to ensure accurate delivery and maximize effectiveness of cell therapeutics in the spinal cord.

References

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3. Gowing G, Shelley B, Staggenborg K, et al. Glial cell line-derived neurotrophic factor-secreting human neural progenitors show long-term survival, maturation into astrocytes, and no tumor formation following transplantation into the spinal cord of immunocompromised rats. Neuroreport. Apr 16 2014;25(6):367-372.

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