



Fate of transplanted bone marrow derived mesenchymal stem cells following spinal cord injury in rats by the transplantation methods.

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Disclosure of potential conflict of interest

YH Kim

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KY Ha

Noting to disclosure.



Prerequisites for Clinical application of Cell therapy

Selection of the ideal cells

Transplantation methods & Dosages

Timing of transplantation

Should be addressed.



The purpose of study

Ideal Transplantation route ?

→ Comparison of transplantation methods

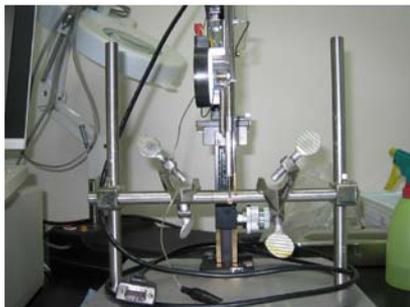
IV (intravenous transplantation) and IL (intralesional transplantation) of allogenic bone marrow derived mesenchymal stem cells (MSCs) following contusive spinal cord injury.



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Materials and Methods



Contusive SCI



Precultured and PKH26 tagged MSCs



Post-injury 1 day

Control : SCI only

IL group: at post-injury day one the injured sites were re-exposed and a concentration of 1×10^6 cells in $10 \mu\text{l}$ was injected using a Hamilton needle.

IV group: PKH 26 tagged 1×10^6 cells in a 0.5ml total volume were injected through the tail vein 24 hrs after SCI.



Materials and Methods (assessment)

- Engraftment of the transplanted cells
- Aberrant Differentiation of the engrafted cells
- Neuronal lineage differentiation of the transplanted cells
 - Expression of Neurotrophic factors
 - Functional improvements (BBB scale)



Results

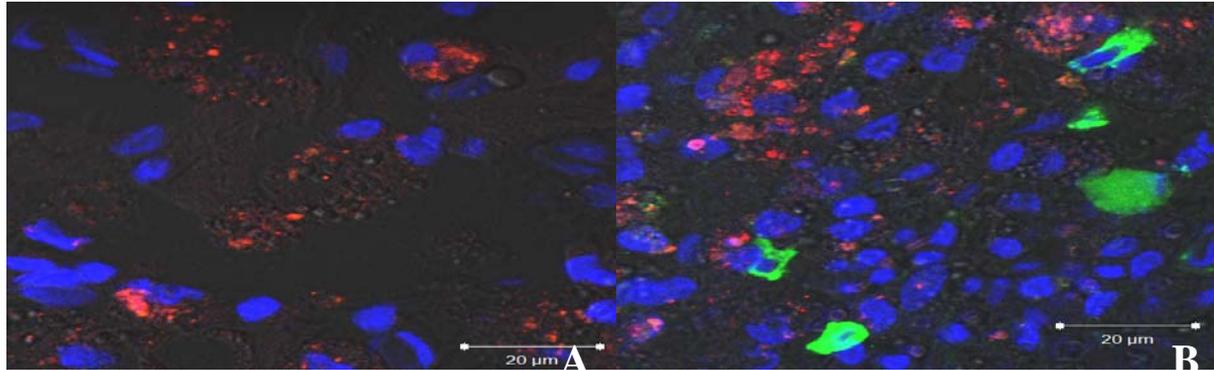
Phenotype confirmation of MSCs using FACS

Flow cytometric analysis of cultured cells with CD 45, 73 and 29.

The positive expression of CD 73 and CD 29 and negative expression of CD 45 indicate its mesenchymal stem cell lineage

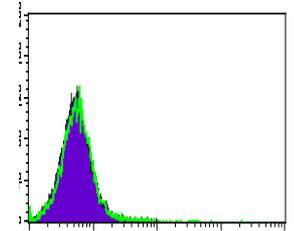
Aberrant differentiation of the transplanted cells

To identify an aberrant differentiation of the transplanted cells to mesenchymal lineage, type II collagen staining was performed.

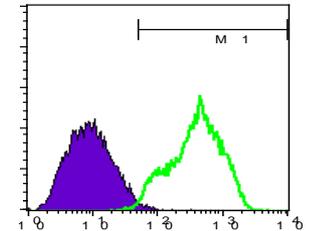


Type II collagen expression of the transplanted cells were observed. In both transplanted groups, cells with a colocalization of PKH 26 and collagen II were not detected. A) The IV transplanted MSCs did not express type II collagen. B) Some type II collagen expression was noted in the IL group, however, no colocalization was found with PKH26 expression (collagen was tagged with green fluorescence, magnification x400, scale bar 20 μ m).

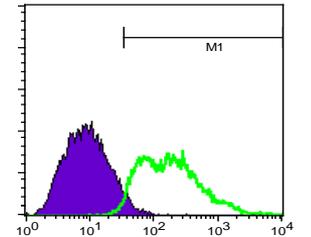
CD 45



CD 73



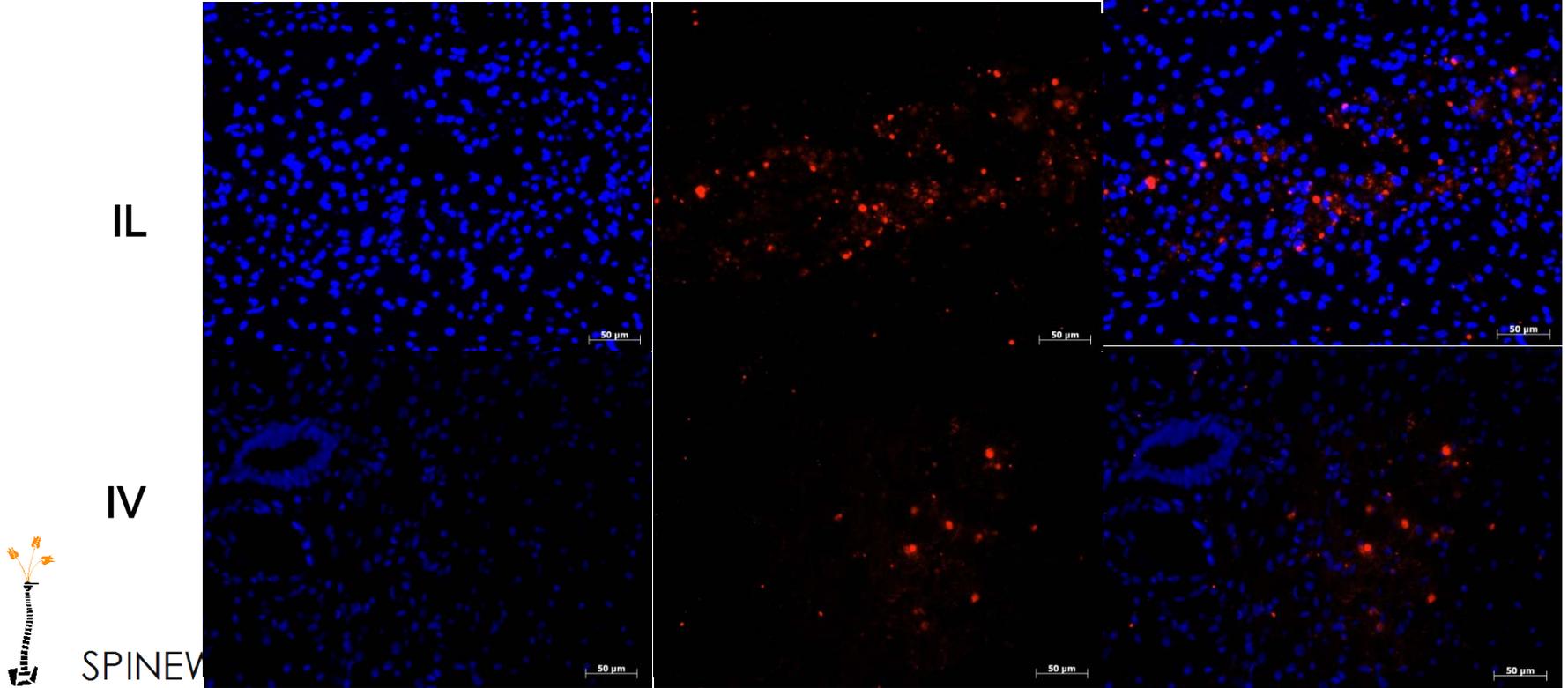
CD 29



Results

Identification of the transplanted cells in vivo

In both treated groups, the transplanted MSCs were found at the posterior portion (injured site) of the spinal cord and some scattered cells were also observed at the gray and white matter adjacent to the injured site.



Results

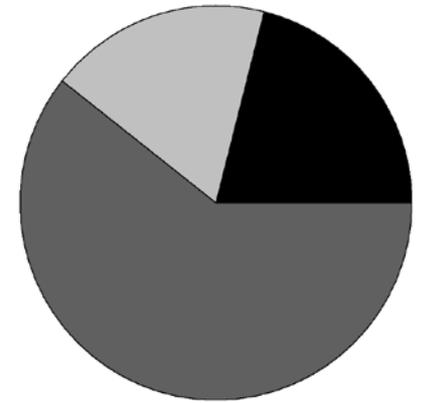
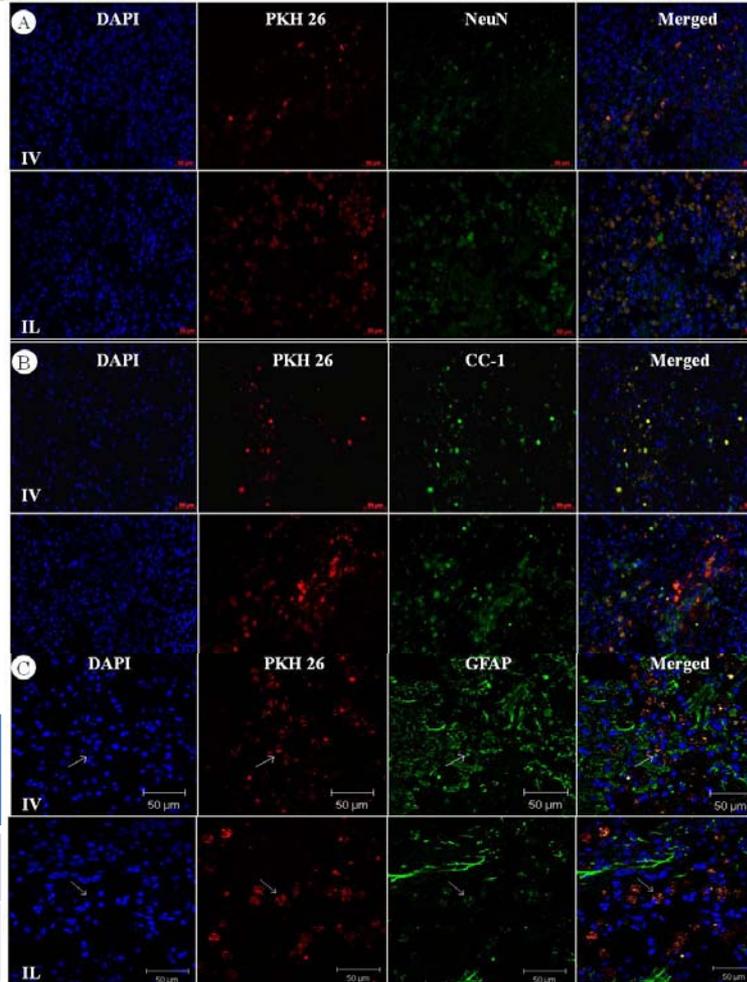
Differentiation of MSCs

Various expressions of neural and glial cell makers of engrafted MSCs. Pkh26 positive cells were mainly found at the injured sites.

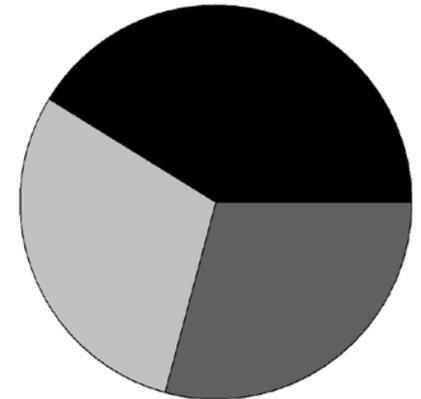
- A) Neuronal differentiation of the transplanted MSCs. (n=4, two tissue samples and six fields in each sample).
- B) Oligodendrocyte differentiation of the transplanted MSCs.
- C) Astrocyte differentiation of the transplanted MSCs.

IV transplanted MSCs were mainly expressed the astrocyte differentiation. The proportion of neuronal and oligodendrocyte differentiation were lower than that of IL transplanted MSCs (magnification x200, scale bar 50 μ m).

	Neuronal differentiation	Oligodendrocyte Diff.	Astrocyte Diff.
IL	9.4 \pm 0.9	8.2 \pm 1.1	27 \pm 1.0
IV	28.4 \pm 2.4	20.4 \pm 1.3	20 \pm 1.3



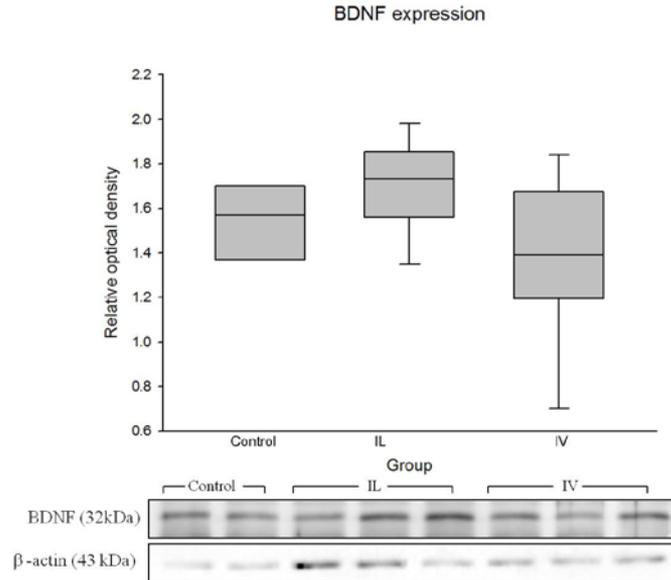
IL transplantation



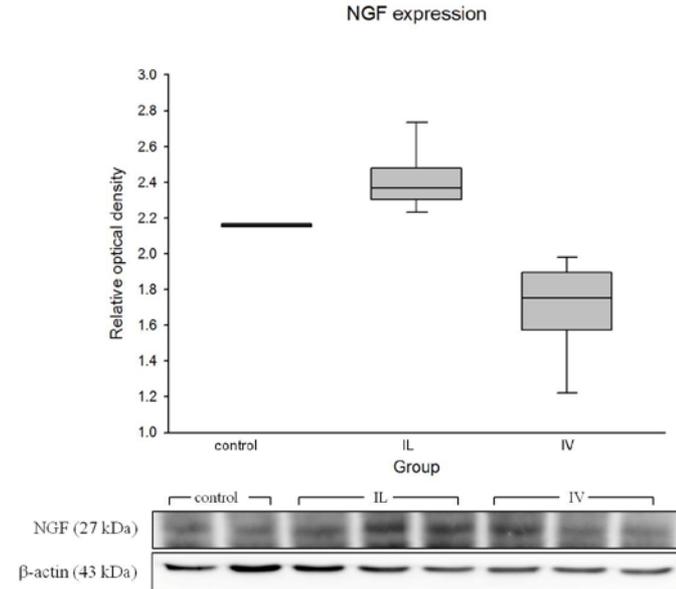
IV transplantation

Results

Neurotrophic factors expression in the SCI lesion



BDNF level in the IL group (mean relative optical density, 1.70 ± 0.2) were slightly increased compared to those in the control group (1.58 ± 0.22) and the IV group (1.39 ± 0.35). However, there was no statistical significance

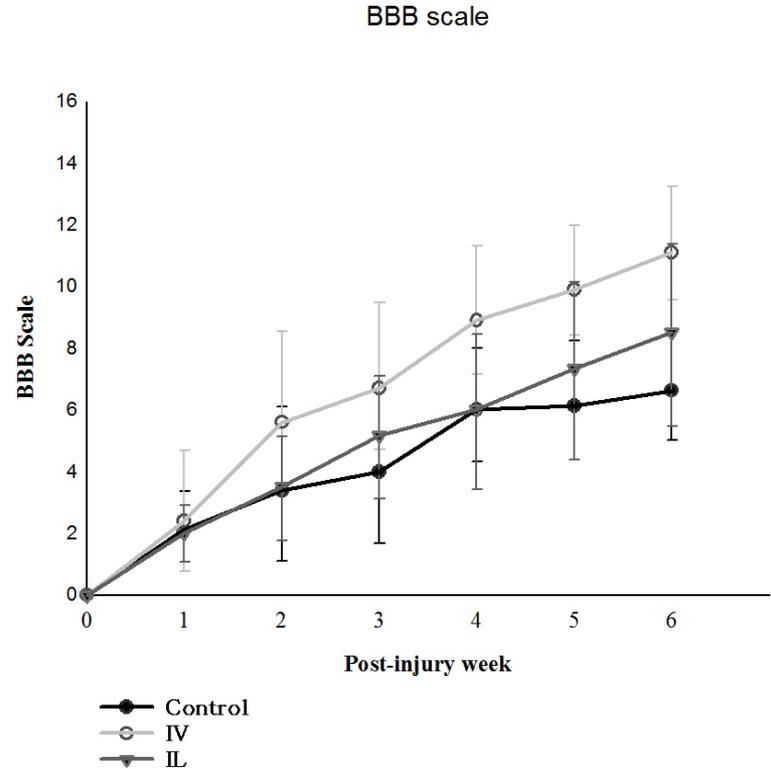


NGF level in the IL group (mean relative optical density, 2.4 ± 0.15) was significantly increased compared to the control (2.16 ± 0.04) and the IV group (1.7 ± 0.23) ($P < 0.05$)

Results

Behavioral assessment

All the injured rats manifested complete hindlimb paraplegia immediately after the operation. In all the groups, the rats gradually recovered varying degrees of motor function over the time of observation (Fig. 6). At 6 weeks post-injury, the mean BBB motor scales in the control, IV and IL groups were 6.5 ± 1.8 , 11.1 ± 2.1 and 8.5 ± 2.8 , respectively. The functional recovery seen in the rats that underwent MSCs transplantation was significantly better than that in the control group ($P < 0.05$).



Conclusion

Although our results could not present any superiority of MSCs transplantation routes for SCI, we could suggest that **early delivery of allogenic MSCs following SCI provided favorable behavioral improvement compared to the control group.**

And fate of transplanted MSCs and expression of neuronal growth factors were different by the transplantation routes.

Ongoing research is needed to identify the mechanism of this differences that observed in this study and whether these different transplantation routes could make differences in changing the milieu of the injured spinal cord.

