Mesenchymal stem cell treatment of traumatic brain injury

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There is now imposing literature from many laboratories that the treatment of traumatic brain injury (TBI), stroke, intracerebral hemorrhage, spinal cord injury, and neurodegenerative disease using mesenchymal stem cells (MSCs) produces functional benefit, although without reducing the lesion, indicating that these cells stimulate the recovery of function and remodel injured tissue. Clinical data supporting these laboratory findings have also emerged. In the study by Harting et al., the authors purport that intravenously administered MSCs have no beneficial functional effects in a model of TBI.

There are, however, a number of basic differences between the study by Harting et al. and those of others that make interstudy comparisons difficult. To visualize the injected cells, Harting and colleagues labeled cells with quantum dots (QTracker 655 and 800). The effects of quantum dots on the function of MSCs—that is, whether they alter the expression of genes and proteins vital for the induction of functional benefit—have not been investigated. Moreover, the efficiency of the detection of these labels in vivo is not known. Studies in which we administered male MSCs to female animals, which provide an unequivocal and clear identification of labeled injected cells, have shown a greatly increased presence of infused cells within parenchymal brain tissue compared with studies in which quantum dots have been used. Furthermore, based on our data, labeling of cells with green fluorescent protein (GFP) in vitro provides a 95% labeling efficiency; however, these cells failed to identify MSCs in vivo. Thus, it is essential to test the effects of the label (quantum dots) on the cell characteristics and to test the efficiency and sensitivity of measurements in vivo before comparisons can be made with other MSC studies.

All cells are not equivalent in terms of their therapeutic benefit. Harting et al. used MSCs from Passages 3–8. We and others have used cells from Passages 1–3, although most of our studies have been performed with Passage 1 MSCs. Cell culturing also alters cell characteristics. Therefore, generalizations regarding the use of specific MSC preparations, for example, those from Passages 3–8, under very different conditions as a treatment for TBI cannot be made. Cells are altered with the passage number, and the exact conditions for cell preparation utilized by others first should be compared with the new cell preparation of Harting et al. before questioning the effects of MSCs on TBI. In addition to the use of very different cells, the experimental conditions in the Harting et al. study were quite different from those in previous reports, particularly from our group. The controlled cortical impact model produced by Harting and colleagues resulted in a modified neurological severity score of 4.5, in contrast to our score of 9.0. A relatively low severity score may obscure the discrimination of a functional benefit, and the animals all showed a rapid return to baseline or near-baseline values. More importantly, Harting et al. killed the animals too early, at 14 days after TBI. We have found functional benefit at ≥30 days after treatment according to a modified Morris water maze test and at ≥15 days after treatment according to other neurological tests. By using additional animals and injuries that show robust deficits and by killing these animals at later time points, Harting and colleagues may notice a functional benefit.

There are also some experimental issues in their study that require clarification. Arterial blood sampling in the animals, which appeared to involve the extraction of blood within 15 minutes, induces approximately 50% blood loss. Did Harting and colleagues replace the blood? If blood was not replaced, vasoconstriction could affect the distribution of MSCs, increasing their presence in the lungs and reducing the arterial concentrations of MSCs.

In summary, the cells and experimental conditions used in the study by Harting et al. were very different from those in previously reported studies, particularly studies by our group. These differences make it very difficult to draw any conclusions. Harting et al. should first seek to replicate the conditions in many studies of MSCs by using well-established cell labeling and preparation methods and experimental conditions before making any conclusions about the efficacy of intravenously administered MSC therapy for TBI.

References

Editorial


Response

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We welcome Dr. Chopp and coworkers’ critique of our work regarding the intravenous delivery of MSCs in a model of TBI in rodents. We have followed the work of his group with interest and awe.

Their queries surround a few fundamental issues in the field: cell labeling (and thus tracking) and cell characterization (that is, “my cells are better than/different from your cells”). They admonish us to first use “well-established cell labeling techniques” in our studies. In fact, we rigorously explored the cell label matter, initially using MSCs isolated from GFP+ transgenic rodents and noting the issue of epigenetic gene silencing of the GFP gene in vitro and in vivo by DNA methylation–mediated mechanisms (unpublished data) (as opposed to histone acetylation). We found that the GFP gene is silenced with cell division, thus making this label rather unreliable for cells that must be expanded in vitro (MSCs) or must divide in vivo. Other techniques have been utilized extensively by Chopp et al., including the use of the thymidine analog bromodeoxyuridine (BrdU), which has led to numerous erroneous conclusions regarding the phenotypical “changes” of MSCs into neural elements.5 Coyne et al.2 and others have noted that these findings are really attributable to the transfer of the label to dividing neural progenitor cells and had nothing to do with the “transdifferentiation” of MSCs into neural phenotypes. Although the sex chromosome label technique for cell transplantation is potentially elegant, it fails to recognize the issue of fetal microchimerism, especially in female rodents that have had multiple litters (they come from breeding houses). In short, female rodents can display cells within the brain containing male chromosomes in varying amounts, which is presumably related to the number of pregnancies (and number of male gestations). Furthermore, in nonlesioned animals, these cells localize to niches thought to house progenitor cells, suggesting that they may have proliferative capacities, which fully explains the findings with the chromosomal label technique.0 Finally, quantum dot toxicity has been investigated in a number of venues, including MSC survival, cytokine production, proliferation, and DNA damage, and has shown minimal toxicity.2 In short, despite the critique, the jury is still out with regard to the best label for progenitor cell biology.

Now, to address the issue of cell characterization, we rigorously immunophenotyped the cells used in our study to avoid the very criticism leveled at us. Specifically, we...