Dual Modality Monitoring of Intracerebral Stem Cell Delivery and Distribution Following Reperfused Ischemia

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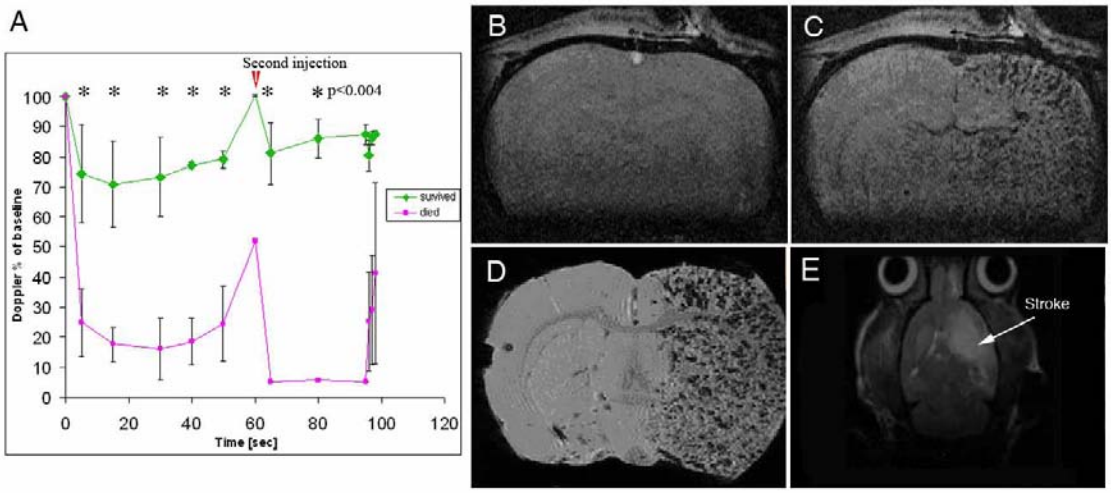
Introduction: Stroke is a leading cause of death and disability, often leaving survivors with permanent neurological deficits and long-term disability. In animal models, it has been shown that neurological deficits after an ischemic insult can be alleviated by injection of stem cells[1]. MR tracking of magnetically labeled stem cells has shown to be a valid technique to monitor stem cell migration in the brain[2,3]. While there is general agreement about beneficial effects of stem cells upon outcome following ischemia, the underlying mechanisms need further clarification. Stem cell injection may result in trophic support or neuronal replacement, and optimization of a robust engraftment is highly desired. While intravenous (IV) and intraparenchymal (IP) injection routes are currently most often used for intracerebral cell delivery, both have limited potential for widespread, targeted delivery to the ischemic lesion, due to either major organ trapping (liver, lung and spleen for IV injection) or tissue inaccessibility (IP injection). Intraarterial (IA) delivery, however, provides the opportunity to deliver cells in close proximity to the lesion. Following IA delivery, cells that lodge in the blood vessels of the penumbra with a compromised blood brain barrier will have the greatest potential of entering the brain parenchyma. However, when grafted in high numbers, stem cells can become a source of micro-emboli, potentially causing further perfusion deficits leading to more severe ischemia or even death. It is therefore highly desirable to monitor precisely the cellular delivery in order to adjust the magnitude of cell delivery effectively. We hypothesized that laser Doppler measurements of cerebral blood flow during IA stem cell injection is a predictor of cellular lodging in cerebral vessels and formation of microemboli, while MRI cell tracking can be used to assess subsequent cell distribution in the brain

Methods: Fisher rat mesenchymal stem cells (MSCs) were labeled with Feridex and BrdU and suspended at $1x10^6$cells/ml. Transient middle cerebral artery occlusion (MCAO) was induced in female Wistar rats (n=17) using the intraluminal suture technique of vessel occlusion for 2 hours. Following reperfusion, the extracranial right internal carotid artery (ICA) ipsilateral to the MCAO was cannulated with plastic tubing. Cells were infused at 1ml/min; after 30 sec the injection was interrupted for 30 sec and restarted for another 30 sec to complete the injection of $1x10^6$cells total. Transcranial Doppler signals were recorded during the entire injection period (LDF; Moor DRT4). For MRI, rats were isoflurane-anesthetized and imaged with a Bruker 4.7T spectrometer using a T2-weighted spin echo sequence (TE/TR=98/1300ms, AV=2, RES=266x343x350µm, scan time 23min) and a T2*-weighted gradient echo sequence (TE/TR=5/300ms, AV=4, RES=83x125x333, Scan time 20min). Four animals were sacrificed on the day of injection for high resolution ex-vivo imaging (Bruker 9.4 T horizontal bore magnet with a gradient echo sequence, TE/TR=5/100ms, AV=4, and RES=84x83x86) and 6 animals imaged in weekly intervals for up to 3 weeks followed by perfusion and histological analysis.

Results: We observed that intraarterial grafting of MSCs does reduce cerebral blood flow as measured by Laser Doppler. Injection of $1x10^6$ MSCs caused rapid death of 30% of animals. Reduction of the Doppler signal below 50% of baseline was correlated with poor survival (A). MRI imaging before (B) and after cell injection (C) confirmed lodging of labeled cells in the right ICA vascular territory, as visualized by the presence of strong signal voids that were further confirmed by high-resolution ex-vivo imaging (D). Anti-BrdU immunostaining of brain tissue was found to correlate with the MRI findings. The iron oxide from injected cells did not interfere significantly with T2-weighted imaging of stroke (E), allowing further quantitative evaluation of the effect of MSC therapy on stroke development.



Conclusions: Laser Doppler is well suited for real-time monitoring of effective intraarterial cell delivery to the brain, while MRI allows detailed assessment of intraparenchymal cell distribution. Continued refinement of these imaging techniques may enhance further development of stem cell-based therapies in stroke.

References:

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