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TECHNICAL NOTE - FUNCTIONAL NEUROSURGERY - OTHER

Technical note: accuracy and precision in stereotactic stem cell transplantation

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Abstract

Background While multiple trials have employed stereotactic stem cell transplantation, injection techniques have received little critical attention. Precise cell delivery is critical for certain applications, particularly when targeting deep nuclei.

Methods Ten patients with a history of ischemic stroke underwent CT-guided stem cell transplantation. Cells were delivered along 3 tracts adjacent to the infarcted area. Intraoperative air deposits and postoperative T2-weighted MRI fluid signals were mapped in relation to calculated targets.

Results The deepest air deposit was found 4.5 ± 1.0 mm (mean ± 2 SEM) from target. The apex of the T2-hyperintense tract was found 2.8 ± 0.8 mm from target. On average, air pockets were found anterior (1.2 ± 1.1 mm, $p = 0.04$) and superior (2.4 ± 1.0 mm, $p < 0.001$) to the target; no directional bias was noted for the apex of the T2-hyperintense tract. Location and distribution of air deposits were variable and were affected by the relationship of cannula trajectory to stroke cavity.

Conclusions Precise stereotactic cell transplantation is a little-studied technical challenge. Reflux of cell suspension and air, and the structure of the injection tract affect delivery of cell suspensions. Intraoperative CT allows assessment of delivery and potential trajectory correction.

Keywords Image guidance · Intraoperative CT · Stem cell · Stereotaxy

Introduction

Stem cell-based treatment for neurological disorders is an evolving but technically challenging field. Stereotactic transplantation of stem cells has been studied for use in multiple conditions including Parkinson's disease [8, 13, 15], Huntington's disease [2], stroke [9], ALS [5], and spinal cord injury [21]. Unfortunately, these early clinical trials have generally not managed to match the success of animal studies [1, 14].

The acquisition and biologic effects of stem cells used for therapeutic transplantation have been studied in depth, but the delivery of stem cells to the central nervous system (CNS) is a complex and little-studied problem [1, 14]. Cell transplantation into the CNS is target-dependent, and the anatomic targets are often small and may neighbor other critical anatomic structures, as is the case in the brainstem and spinal cord. Stereotaxy has therefore been employed for precise stem cell delivery to the CNS, but the accuracy of stereotactic stem cell transplantation has been questioned and there is little data to characterize the distribution of transplanted cells in the human brain.

The present study takes advantage of a randomized controlled trial of mesenchymal stem cell transplantation for ischemic stroke, including the use of intraoperative CT and acute postoperative MRI, to describe the accuracy of stem cell delivery. While other studies have reported on qualitative features of cell delivery, the present study is one of the first to quantitatively assess the accuracy of stereotactic stem cell transplantation immediately post-transplantation in human patients, and is the only report to describe intraoperative CT guidance for stereotactic stem cell transplantation.

This article is part of the Topical Collection on *Functional Neurosurgery - Other*

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Materials and methods

Study design and population

Data were obtained from participants in a trial of stem cell transplantation in patients with ischemic stroke with fixed motor deficit. The acquisition of these data, and the trial as a whole, underwent institutional review (IRB#15-1409). This trial included experimental (stereotactic stem cell injection of stem cells, at two different concentrations) and control (sham surgery with no injection) arms. This study is limited to participants in the experimental arms (both cell concentrations) who underwent transplantation at a single center, with the use of intraoperative CT.

Stereotactic stem cell transplantation

Participants underwent stereotactic frame (CRW, Integra, Hornell, NY, USA) placement under local anesthesia. Following frame placement, a cranial CT with 1-mm slice thickness was obtained using a 32-slice portable CT scanner (BodyTom(R), Neurologica, Danvers MA USA). Using an intraoperative neuronavigation platform (StealthStation S7, Medtronic, Minneapolis, MN, USA), the intraoperative CT was fused to a preoperative contrast-enhanced MRI with 1-mm slice thickness. Three implantation trajectories were designed to maximize cell deposit in the white matter tracts immediately adjacent to the infarcted brain (hereafter referred to as the stroke cavity).

In patients undergoing cell transplantation (i.e., all patients included in the present study), a single burr hole was fashioned at a common entry point from which all three targets could be reached. A straight cannula (0.8 mm outer diameter), attached to a Hamilton syringe containing the stem cell suspension, was stereotactically inserted until the target was reached. Cells were deposited at 5 points along each trajectory, separated by 5 mm, starting at target. At each point, 20 μL of cell suspension was injected over 2 min. Following the final injection, the small dura defect was covered with Gelfoam, the incision was closed in a standard manner, a post-procedural intraoperative CT was obtained under stereotactic conditions with the frame still in place, the stereotactic frame was removed, and the patient was brought to the recovery area. A brain MRI was obtained the day after surgery according to study protocol including T1-, T2-, and diffusion-weighted sequences.

Comparison with stereotactic biopsy

To assess the role of stereotactic brain cannulation versus injection of cell suspension, we examined an additional 2 patients undergoing frame-based stereotactic brain biopsy. Biopsies were obtained through a straight cannula (1.6-mm outer diameter), and a titanium marker was left at the biopsy target. Intraoperative CT was obtained pre- and post-biopsy. Postoperative MRI was not obtained in patients undergoing brain biopsy.

Data extraction and analysis

The post-procedural intraoperative CT and postoperative MRI were fused with the preoperative MRI and pre-procedural intraoperative CT. The three-dimensional coordinates of the targets were obtained from the planned trajectories. On the intraoperative CT, the coordinates of the deepest (i.e., furthest from entry point along injection trajectory) air deposit were recorded. The T2-hyperintense injection tracts were identified on the postoperative MRI, using the preoperative MRI to differentiate postoperative changes from preexisting encephalomalacia (Fig. 1). The coordinates of the deepest point along the visualized injection tract were recorded. The distance from target to the deepest air deposit (CT) and the deepest cell suspension deposit (on T2-weighted MRI) was compared to 0 via a one-sample *t*-test using GraphPad Prism (GraphPad Software Inc., La Jolla CA USA). Similarly, the distance between deepest air and cell suspension deposits was compared to 0 via a one-sample *t* test.

Results

Ten patients underwent cell transplantation. All infarcts were located in the subcortical pyramidal tract proximal to the internal capsule. In most cases, the selected trajectories had a shared frontal entry point and were caudally oriented with anterior, middle, and posterior tracts (as illustrated in Fig. 1). No hemorrhage or other acute pathology was noted on intraoperative CT or postoperative MRI.

Qualitative assessment of the intraoperative CTs revealed significant variation in the location and distribution of air deposits. Air was noted at target in some tracts and above target in others. Multiple foci of air were noted along some tracts (Fig. 2). Air deposits were often seen in the stroke cavity, particularly in tracts that came close to or traversed the stroke cavity (Fig. 3).

On average, the deepest deposits of air and cell suspension were found above target along the injection tracts. The deepest air deposit was located 4.5 ± 1.0 mm (mean ± 2 SEM, $p < 0.001$) from target (Fig. 4). Air deposits were found anterior (1.2 ± 1.1 mm, $p = 0.04$) and superior (2.4 ± 1.0 mm, $p < 0.001$) to target. The deepest cell suspension deposit was located 2.8 ± 0.8 mm from target ($p < 0.001$). No directional bias (anterior-posterior, medial-lateral, or superior-inferior) was noted for the apex of the deepest cell suspension deposit relative to target (Fig. 4) or relative to the deepest air deposit (Fig. 5). The deepest air deposit was on average located above the deepest cell suspension deposit (5.1 ± 1.1 mm, $p < 0.001$).

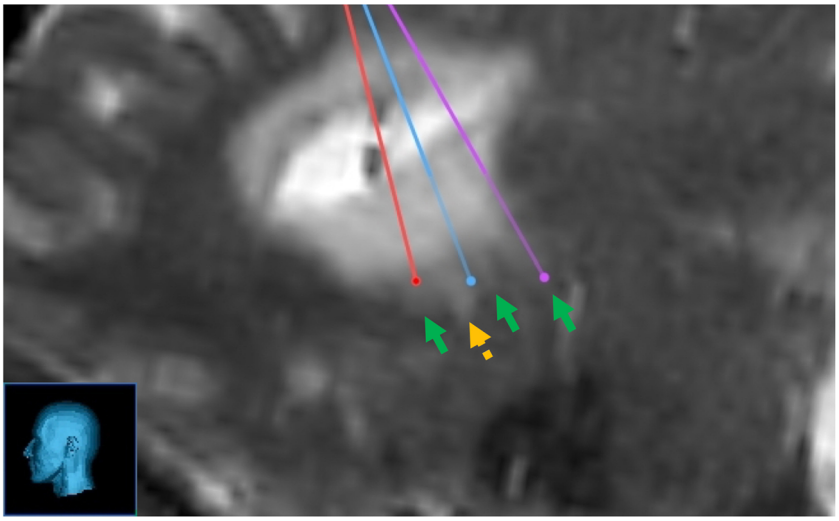

Fig. 1 Visualization of cell suspension tracts on T2-weighted MRI. Cell suspension (solid arrows) can be differentiated from encephalomalacia (dashed arrow) by comparison to preoperative MRI

Compared to patient undergoing stem cell transplantation, patients undergoing frame-based stereotactic brain biopsy were found to have minimal to no intracerebral air along the biopsy tract (Fig. 6).

Discussion

The present study provides some of the first clinical data to support the existence of significant reflux of infusate (and hence of cells) along the injection tract. Cell suspension (as visualized on T2-weighted MRI) was found near but not always at target. Intracerebral air, while conceptually more prone to reflux than cell suspension, was often found proximally near injection tracts in the stroke cavity. In this particular application, where the goal was to deliver stem cells to the interface between chronic infarct and normal brain, cell reflux into the stroke cavity can significantly decrease the delivery of valuable cell volume to target. The proclivity of cell suspension to reflux along the injection tract supports the use of intraoperative imaging when precision is critical [18].

The use of straight cannula-based injection techniques has been identified as a possible source of error in prior disappointing trials of stem cell transplantation into the human CNS [6, 10, 13]. Most clinical studies (including the present study) deliver stem cells via a straight cannula, mimicking animal studies [1]. Potts et al. [14] recognized this as a problem of scale; the human brain is a thousand times larger than the rodent brain [11], which creates a fundamental difference in the way the stem cell suspension is delivered to the brain parenchyma. The human brain requires larger volume of cell suspension to be injected, often resulting in an increased injection rate and need for multiple cannula passes [1, 14]. The low elasticity of brain parenchyma does not tolerate large or rapid infusion volumes, and the injection tract acts as the path of least resistance, resulting in reflux of cell suspension along the tract and hampering cell suspension delivery to the target [1, 14, 20]. However, there is little human data to clearly describe this phenomenon. Histological analysis of small number of deceased trial participants showed cell survival and

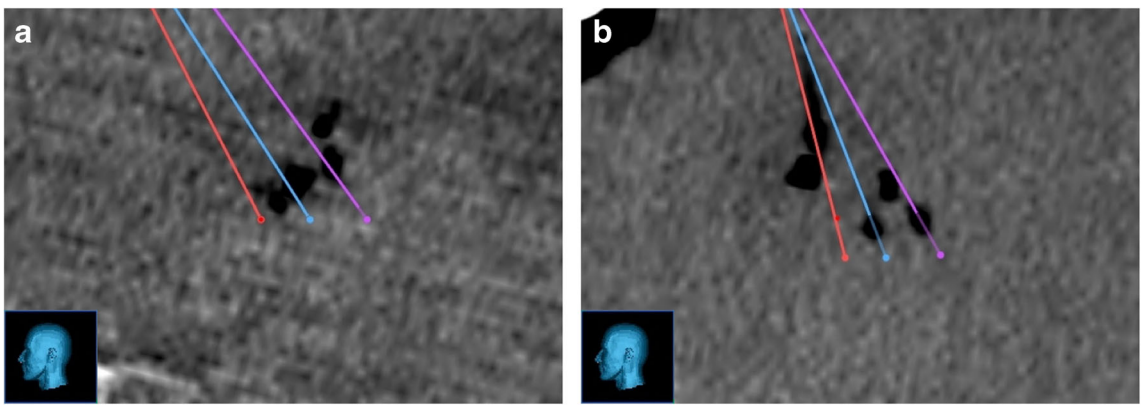

Fig. 2 Variation in intracerebral air deposits seen on CT in two patients (**a** and **b**)

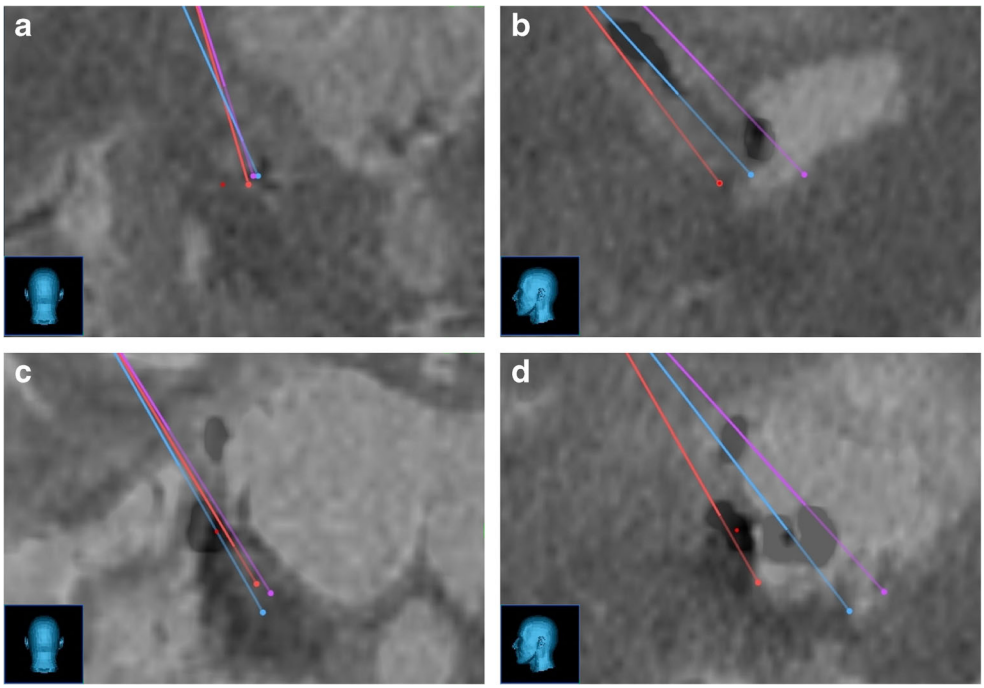

Fig. 3 Air can accumulate in stroke cavity and along the injection tract. Fused postoperative CT and MRI are shown for two patients (**a**, **b**, and **c**, **d**)

growth [8, 15], but the time interval between transplantation and death significantly limits assessment of surgical cell delivery.

Multiple strategies have been suggested to improve targeting error associated with traditional injection techniques. Convection-enhanced delivery can improve the accuracy and delivered volume of brain infusate, but its use in cell transplantation is limited by the relatively large size of cells [19]. Hydrogels have been used in lieu of liquid cell suspensions to improve targeting as well as cell survival [17]. Alternatives to the straight cannula have been developed; for example, a branched deployment device, which allows multiple small-volume radial deposits of cell suspension, has been used without any appreciable cell reflux [16].

This study has important limitations. First, cell suspension deposition was assessed by proxy—via air deposits and iatrogenic T2-hyperintense signal. As previously mentioned, air can be expected to reflux more than fluid. While we refer to the T2-hyperintense tract

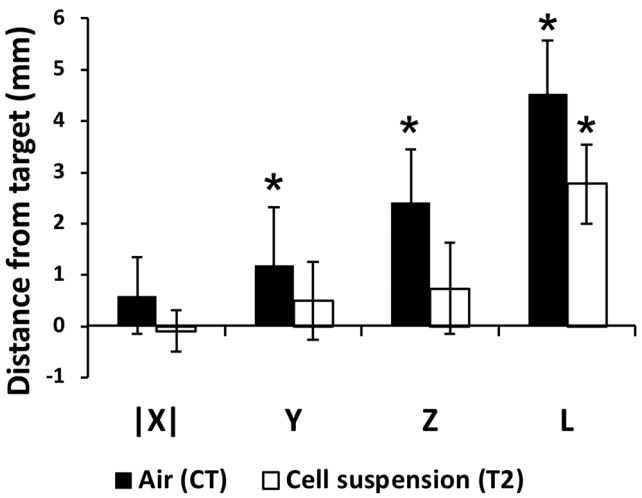

Fig. 4 Distance from deepest air deposit (on CT) and from deepest cell suspension deposit (on T2-weighted MRI) to target. $|X|$ = absolute value of difference in X-axis. L = Euclidean distance. $^*P < 0.05$

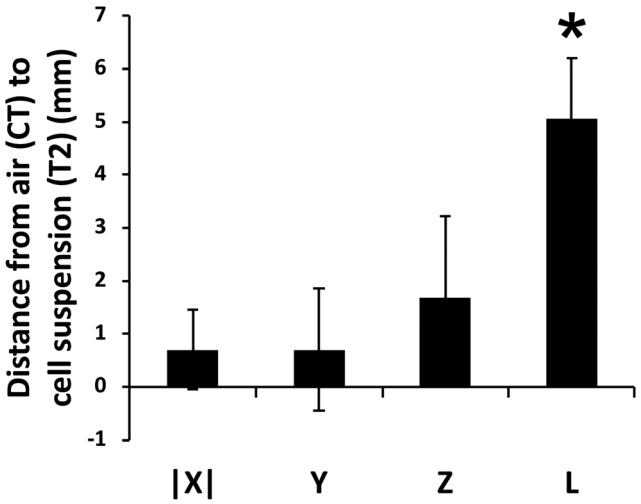

Fig. 5 Distance between deepest air deposit and deepest cell suspension deposit. $|X|$ = absolute value of difference in X-axis. L = Euclidean distance. $^*P < 0.05$

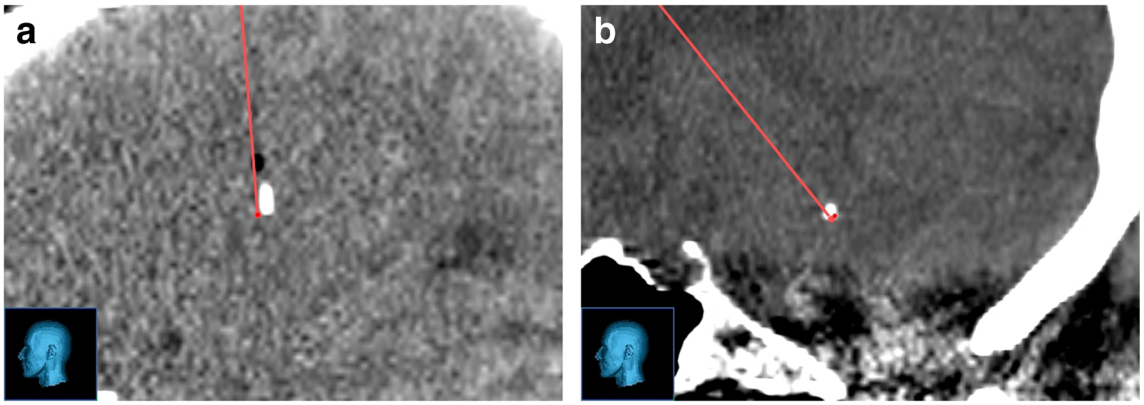

Fig. 6 Intracerebral air deposits are **a** minimal or **b** absent in other frame-based stereotactic procedures such as brain biopsy. The patient in **a** underwent biopsy of a basal ganglia lesion. The patient in **b** underwent biopsy of a pontine lesion. The metal artifact at target corresponds to a titanium marker left at the biopsy site

as a marker of cell deposition, we cannot prove this direct correspondence. New postoperative T2 hyperintensity could also reflect edema associated with cannula insertion, but if this were the case, the T2 signal would be expected to terminate at or just past target, rather than consistently proximal to target. Additionally, if the new postoperative T2 signal reflected traumatic/insertional edema, similar signal should have been seen along the entire cannula trajectory, but this was not observed. For comparison, we examined patients subject to frame-based brain cannulation for the purpose of biopsy. Despite larger cannula diameter, minimal or no intracerebral air deposition was noted on post-procedural CT. Postoperative MRI is not routinely obtained in patients undergoing brain biopsy at our institution, so we could not assess for the presence of procedure-related T2 hyperintensity.

A second limitation is measurement error introduced by stereotactic cannula placement, CT acquisition, and CT-MRI fusion. Targeting errors of 1.1–1.6 mm have been reported for frameless stereotaxy with 8-slice portable CT, while frame-based stereotaxy is associated with higher accuracy, and the use of a 32-slice portable CT scanner (as in this study) would theoretically result in similar or less error compared to an 8-slice scanner [3, 12]. These average errors are small compared to the distance-from-target values reported in the present study.

In summary, this study is one of the first to characterize the accuracy and precision of stereotactic cell transplantation into the human brain in the acute post-transplant period. Though the sample size was small and cell suspension was identified indirectly, these data support the notion that traditional straight cannula-based injection methods (as used in major clinical trials) are associated with reflux-related inaccuracy. Further research might utilize radiographic labeling of transplanted cells (e.g., by use of ferromagnetic particles or MRI-detectable fluorine isotopes) [4, 7, 16] to further describe the distribution of stereotactically transplanted cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the University of Chicago Institutional Review Board (IRB#15-1409) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Comments

This is very interesting study evaluating a technique for precise stereotactic stem cell transplantation in humans after ischemic stroke. The study is well-written with appropriate methodology and meaningful conclusions. This study adds to the important and growing body of work regarding stem cell therapy in humans after neurological injury. The authors are congratulated for their efforts, and I look forward to further work from them in this important area.

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