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Optimized Cannula Design and Placement for Convection-enhanced Delivery in Rat Striatum

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Abstract

The stereotactic delivery of therapeutic agents into brain has been problematic because of reflux and leakage of the delivered agent. Good distribution of infusates by convection-enhanced delivery (CED) depends very much on cannula design, precise cannula placement and infusion rates. We have recently published cannula targeting data for the non-human primate (NHP) putamen in which we defined infusion parameters referred to as “red”, “blue”, and “green” zones for cannula placements that result in poor, sub-optimal and optimal volumes of distribution (Vd), respectively. Therefore, we applied our observations in NHP putamen to the rat brain. Initially, trypan blue dye was infused into agarose gels to evaluate distribution and reflux characteristics of a scaled-down cannula without step and 1-mm stepped cannula. “Stepped” means a sharp transition from a wider stent to a narrower tip; thus the distance of the cannula tip to the larger diameter attachment defines the step distance. Reflux was contained with the stepped design even with an infusion rate of 3.0 μ l/min and large infusion volumes in the agarose gel study. Infusions of a recombinant growth factor, GDNF, into rat striatum demonstrated that the presence of a 1-mm stepped cannula prevented reflux and resulted in excellent distribution of GDNF in the striatum. We conclude that a stepped cannula with a 1-mm tip is important for achieving reliable distribution of infused agents in rat brain. It should be considered when local therapies such as gene transfer, local protein administration or cellular replacement are evaluated in rodent models.

Keywords

MRI; convection-enhanced delivery; stepped cannula; striatum; rat

Introduction

Convection-enhanced delivery (CED) has been developed as a drug delivery strategy and represents a powerful methodology for targeted therapy in the fields of neurodegenerative diseases, such as Parkinson’s disease (PD) [1], and neuro-oncology [2] [3]. Although Infusion of therapeutic agents into the brain by CED has improved, their distribution in the targeted site has been problematic [3]. In particular, reflux and leakage can occur during CED to increase the risk of affecting adjacent regions. The flow of therapeutic agents into the cerebrospinal

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fluid (CSF) is potentially quite serious as it can undermine the efficacy of CED and cause toxicity due to exposure of remote regions of the brain to therapeutic agent [5]. This is an especially important concern when delivering viral vectors that might transduce untargeted cells under such circumstances. It has been reported that onset of chemical meningitis after CED was due to drug leakage in a clinical trial of paclitaxel for treatment of malignant gliomas [6]. Thus, side effects caused by reflux and leakage of administered agents may prevent safe delivery of a therapeutic agent via CED. These events have limited a broader clinical application of CED.

During CED, the volume of distribution (V_d) for a given agent depends on the structural properties of the tissue being convected, such as hydraulic conductivity, vascular volume fraction, and extracellular fluid fraction [4]. It also depends on the technical parameters of the infusion procedure such as cannula design, cannula placement, infusion volume, and rate of infusion [5] [6] [7], with the overall aim of improving delivery efficiency while attempting to limit the spread of the therapeutic into regions outside the target. In our experience, a key component of successful CED is likely to be the site of cannula placement within the targeted area, such as putamen [8]. The distance from cannula step to corpus callosum (CC) was found to be critical for optimal distribution of therapeutics. Selection of the optimal cannula type for effective CED delivery in the brain is also critical. We examined several types of cannula with respect to size and design and concluded that a stepped design with a fused silica tip provided us with the most consistently robust brain delivery [9] [10]. The stepped cannula dramatically reduces reflux along the infusion device by restricting initial backflow of fluid flow beyond the step. To obtain effective distribution of the infused therapeutic within the striatum of rats, it is essential to understand the optimal site of placement of the cannula step and tip within these targets and surrounding white matter tracts that serve as leakage points. Such an understanding may allow precise delivery of the therapeutic to the targeted structures while lessening the risk of affecting adjacent regions. In the present work, we have applied the concept of RGB zones, previously developed to analyze putaminal CED infusions in non-human primate (NHP), to CED in the striatum of rats. RGB zones (red, green and blue zones) are the defined areas in the putamen of NHP for cannula placement during CED, which are significantly related to the resultant distribution of infusate [8]. Our findings suggest that the silica cannula with a 1-mm step prevents reflux and maximizes the distribution of agents delivered in the striatum in rats if the cannula is placed properly [8].

Materials and Methods

Cannula design

Two types of cannula were used in this study: (i) a cannula with a 1-mm step and (ii) a cannula without a step. Cannulae without steps were made directly from flexible silica tubing with an inner diameter (ID) of 99 μm and an outer diameter (OD) of 236 μm (Polymicro Technologies, LLC, Phoenix, AZ). Cannulae with a 1-mm step consisted of a step made from silica tubing with an ID of 319 μm and an OD of 431 μm (Polymicro Technologies, LLC, Phoenix, AZ) with glued-in fused silica tubing having ID of 99 μm and an OD of 236 μm . The fused silica tubing was cut by diamond cut at 1 mm for the rat study, as measured from the cannula step. The term "stepped" refers to the sharp transition from the wider stent to a narrow tip (Fig.1); thus the distance of the delivery cannula tip to the larger diameter attachment defines the step distance.

The gel model

CED of trypan blue dye in agarose gel has previously been described as a model to simulate CED in brain [11] [10] [12]. We used 0.25% agarose gel (Life Technologies, Inc., Gaithersburg, MD) in phosphate-buffered saline for all experimental gel studies. Briefly, the infusion system

consisted of a fused silica cannula that was connected to a loading line (containing trypan blue dye), an infusion line with oil and another infusion line with trypan blue solution. A 1-ml syringe (filled trypan blue solution) was mounted onto a micro-infusion pump (BeeHive, Bioanalytical System, West Lafayette, IN), regulated the flow of fluid through the system. The cannula with 1-mm step or without step was fixed with a holder attached to a stereotactic frame to minimize irregularities. The tip of the cannula was placed at a depth of 20 to 25 mm in the gel, and CED of trypan blue solution (0.4%, Sigma-Aldrich Corp., St Louis, MO) was begun. The total infusion volume was 79 μ l. We used the same infusion parameters for every gel study. Infusion rates were as follows: 0.1 μ l/min was applied when lowering cannula to targeted area and increased at 10-min intervals to 0.2, 0.5, 0.8, 1.0 μ l/min and up to 2.0 μ l/min. Each condition with each cannula was tested at least three times to assess reflux. All pictures were taken with a digital camera (Canon SD-100; Canon U.S.A., Inc., Lake Success, NY) at different time points during CED.

Animals

Six male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA; 250 to 300 g at the start of the study) were housed in the laboratory animal research center at University of California San Francisco. The rats were kept on a 12-hour light/dark cycle, and housed individually with free access to food and water. Experimentation was performed according to the National Institutes of Health guidelines and to the protocols approved by the Institutional Animal Care and Use Committee at the University of California San Francisco (San Francisco, CA).

Infusion of GDNF into striatum of rats

Under surgical anesthesia (2% isoflurane, Aerrane; Ohmeda PPD, Inc., Liberty Corner, NJ; 2 l/min O₂), animals were placed in a small-animal stereotactic frame (David Kopf Instruments, Tujunga, CA). A sagittal incision was made through the skin to expose the cranium, and a burr-hole was made in the skull at 0.5 mm anterior and 3 mm laterally from the bregma by means of a small dental drill. Infusions were performed at a depth of 5 mm from the brain surface by the CED method as described previously [12] [13]. Cannula with a 1-mm step or without step (as used in agarose gel study) were connected to 1-ml gas-tight Hamilton syringes (Hamilton Co., Reno, NV) that were attached to the stereotactic frame with a holder. Recombinant human glial cell derived neurotrophic factor (GDNF, 0.2 mg/ml in PBS, R&D Systems, Inc., Minneapolis, MN) was infused at a sequential, ascending infusion rate (0.2 and 0.5 μ l/min) over a 13-minute period with a total volume of 5 μ l. After infusion, animals were euthanized and perfused for immunohistochemistry.

Immunohistochemistry

To detect expression of GDNF in the brain, the rats were sacrificed after infusion of GDNF into putamen. The animals were perfused with saline, followed by 4% paraformaldehyde. Brains were post-fixed overnight in 4% paraformaldehyde, followed by 30% sucrose. Frozen brains are cut into 40- μ m sections on a MICROM HM450 sliding microtome (Fisher Scientific, Philadelphia, PA). Sections were blocked in 2% normal horse serum followed by incubation overnight at room temperature with primary goat anti-human GDNF (R&D Systems, Inc., Minneapolis, MN) diluted in 1:500. Secondary horse anti-goat IgG was used to detect the primary antibodies. Immunoreactivity was visualized with DAB working solution according to the supplier's recommendations (Vector Laboratories, Inc., Burlingame, CA). The negative controls omitted primary antibody. Pictures of GDNF staining were taken with a magnification of $\times$ 1.25 under identical conditions.

Volumetric quantification of distribution of GDNF

Brain sections were used for volumetric quantification of distribution of GDNF. The distribution volume (Vd) of GDNF in the brain of each subject was quantified on an Apple Macintosh G4 computer with the Image J program (Image Processing and Analysis in Java). Regions-of-interest (ROI) derived in the striatum, corpus callosum (CC) and white matter track (WMT) were manually defined, and software then calculated the area from each brain section, and the volume of the ROI was then calculated as area defined multiplied by slice thickness. The boundaries of each distribution were defined in the same manner in series of brain sections.

Statistical Analysis

The volume of distribution (Vd) of GDNF in the striatum as a percentage of the entire putamen volume was compared across subject groups by Student's t-test. The criterion for statistical significance for all tests was $p < 0.05$.

Results and Discussion

First, cannulae without step were evaluated, and the flow rate and infusion volume at which reflux occurred with each cannula were noted under the conditions described above. Silica tubing without any step allowed some distribution of trypan blue in agarose gel but reflux occurred at a flow rate of only 0.5 $\mu\text{l}/\text{min}$ with a consequent small infusion volume of 4 μl (Fig. 2A). As infusion rate and volume increased, reflux enlarged and extended to the top of agarose gel (Fig. 2B and 2C).

In the next series of experiments, reflux was assessed in agarose for the stepped cannula with fused silica tubing. Based the concept of RGB zones for cannula placement in NHP putamen, the tubing was cut 1-mm distal from the cannula tip to adjust the device to the size of the rat brain because our measurements showed that the distance from the upper border to the lower border of rat putamen was only 3 – 3.5 mm. The longer tips used in NHP are, therefore, problematic in the smaller rat brain. For example, if a cannula with a 2-mm step (Fig. 3A) were used for CED in the striatum of rats or the 1-mm step of cannula was placed too close to CC (Fig. 3B), the critical distances from cannula step to CC would be not preserved, which would increase the possibility of reflux and leakage. Therefore, we investigated whether a 1-mm stepped cannula, that preserves the critical distance from step to CC, would lead to more effective CED in the rat striatum in a manner analogous to the optimization previously established in NHP striatum [8]. The infusion rates used for delivery were the same as those used for the cannula without a step. No reflux occurred with the stepped cannula, even at the highest infusion rate of 3.0 $\mu\text{l}/\text{min}$ and large infusion volume of 79 μl (Fig. 2D–F). The distribution of trypan blue was spherical under the conditions used.

In rats, cannulae with and without 1-mm step were placed into right and left striatum respectively (Fig. 4A). Minor tissue damage was found by H&E staining at the site where stepped cannula was placed (Fig. 4B–4D). The infusion rates and delivery times were 5 min at 0.2 $\mu\text{l}/\text{min}$ and 8 min at 0.5 $\mu\text{l}/\text{min}$. Both hemispheres were infused with 5 μl of GDNF ($N = 6$) to assess infusate distribution in the striatum and possible reflux and leakage into surrounding structures in the brain. In our experience, high infusion rates (not used in this study) resulted in reflux and leakage into adjacent area while reducing desired Vd in the target.

The cannula with 1-mm step prevented reflux or leakage in the rat brain at the infusion rates used. At the end of infusion, the cannula was left in place for 5 min before it was withdrawn, but small amounts of GDNF appeared to follow the track left by the cannula as the device was removed. Excellent Vd of GDNF was obtained in the putamen, ranging from 34.8 to 57.9 mm^3 with mean volume of $45.4 \pm 3.1 \text{ mm}^3$ (Fig. 5A). Slight leakage of GDNF into white matter

tracts (WMT) and CC was found in three cases, and their Vd in WMT ranged from 0.8 to 2.6 mm³ with mean volume of 1.6 ± 0.3 mm³ (Fig. 5A).

In rats (N = 6) infused by cannula without a step, relatively poor distribution of GDNF in the striatum was obtained, ranging from 16.6 to 33.6 mm³ with mean volume of 24.3 ± 3.6 mm³ (Fig. 5A). All 6 cases were found to have significant leakage into CC and WMT, and the Vd of leakage ranged from 3.1 to 14.8 mm³ with mean volume of 10.9 ± 2.7 mm³ (Fig. 5A).

Figure 5B shows the percentage of Vd of GDNF in the striatum vs. total Vd in striatum, WMT and CC based on cannula types. When the 1-mm step cannula was used and placed properly, the Vd percentage in the striatum ranged from 94.5% to 100% with mean of $97.2\% \pm 1\%$, indicative of very little leakage of GDNF into the CC. However, even with correct placement of the step-less cannula resulted in a Vd percentage in the striatum between 60.1% to 84.2% with mean of $70.7\% \pm 5\%$ with significant leakage into the CC. Representative GDNF staining shows distribution of GDNF in the striatum and WMT in Fig. 6. These results suggest that better distribution of GDNF in rat brain may be obtained if a cannula with 1-mm step is used. These findings define an optimal cannula design that, together with correct placement within the target regions (striatum in this case), directs excellent distribution of delivered agent into rat brain, concomitantly avoiding leakage into surrounding structures.

In the present study, we evaluated the usefulness of a stepped design of cannula modified to suit the smaller rat brain by shortening the cannula tip from 3 mm as used in non-human primate studies [9] to a more appropriate 1 mm in order to clear the boundary of the CC. In the agarose gel study, the step-less cannula allowed reflux along the cannula occurred at only 0.5 µl/min with a correspondingly small infusion volume of 4 µl. Moreover, in an in-vivo study in rats, only partial distribution of GDNF was obtained with a Vd percentage of $70.7\% \pm 5\%$ in the striatum, and significant reflux and leakage into CC and WMT. In contrast, agarose gel experiments with the 1-mm step cannula no reflux occurred even at a high infusion rate of 3.0 µl/min, achieving a large infusion volume of 79 µl in agarose gel. Similarly, an in-vivo study in rat demonstrated excellent distribution of GDNF in striatum with Vd coverage of $97.2\% \pm 1\%$ with the 1-mm step cannula and little evidence of reflux. Given that the rat-specific stepped cannula directs excellent distribution of delivered agents within the targeted site, with greatly minimized reflux and leakage into surrounding anatomical structures, we would argue that this design should become a standard part of any rodent study in which broad but controlled distribution of infused therapeutic agents is required.

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Figure 1.

Cannula without step and with 1-mm step. Cannulas were made of silica tubing with an inner diameter (ID) of 99 μm and an outer diameter (OD) of 236 μm . One cannula with a 1-mm step consisted of a step made from silica tubing with an ID of 319 μm and an OD of 431 μm out of thinner silica, called stepped cannula. CC, corpus callosum; Str, striatum; WMT, white matter tract.

Figure 2. Distribution of trypan blue in the agarose gel by CED. Panels A – C show CED with cannula minus step. Panels D – F show CED with cannula plus 1-mm step.

Figure 3.

Based on the concept of RGB zones for cannula placement in NHP putamen, if cannula with 2-mm step (A) was used for CED in the striatum of rats or the 1-mm step of cannula was placed too close to CC (B), the cortical distances from cannula step to CC would not be preserved, which would increase the possibility of reflux and leakage. However, if 1-mm step cannula were placed properly, excellent distribution of infusate would be obtained (C). CC, corpus callosum; Str, striatum; WMT, white matter tract.

Figure 4.

Cannula placement in the striatum of rat brain (A). Minor tissue damage was found by H&E staining at the site where cannula was placed (C). **B**, Left side, cannula without step. **C**, Right side, cannula with step. **D**, High magnification image of the tissue at the cannula placement site. CC, corpus callosum.

Figure 5.

Distribution of infused GDNF in rat brain. (A) Volume of distribution of GDNF in striatum and leakage into white matter track (WMT) and corpus callosum. **, $p < 0.001$. (B) Percentage of Vd of GDNF in the striatum vs. total Vd in striatum and white track matter based on the types of cannula. **, $p < 0.001$. Recombinant human glial cell derived-neurotrophic factor (GDNF, 0.2 mg/ml in PBS) was infused at a sequential, ascending infusion rate (0.2 and 0.5 μ l/min) over a 13-minute period with a total volume of 5 μ l.

Figure 6.

Representative GDNF staining in the rat brain. Cannula with 1-mm step was placed in the right striatum, and step-less cannula was placed in left striatum. Recombinant human glial cell derived-neurotrophic factor (GDNF, 0.2 mg/ml in PBS) was infused at a sequential, ascending infusion rate (0.2 and 0.5 μ l/min) over a 13-minute period with a total volume of 5 μ l. CC, corpus callosum; Str, striatum; WMT, white matter tract; w/o, without.