Minimally invasive convection-enhanced delivery of biologics into dorsal root ganglia: validation in the pig model and prospective modeling in humans

Technical note

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Dorsal root ganglia (DRG) are critical anatomical structures involved in nociception. Intraganglionic (IG) drug delivery is therefore an important route of administration for novel analgesic therapies. Although IG injection in large animal models is highly desirable for preclinical biodistribution and toxicology studies of new drugs, no method to deliver pharmaceutical agents into the DRG has been reported in any large species. The present study describes a minimally invasive technique of IG agent delivery in domestic swine, one of the most common large animal models. The technique utilizes CT guidance for DRG targeting and a custom-made injection assembly for convection-enhanced delivery (CED) of therapeutic agents directly into DRG parenchyma. The DRG were initially visualized by CT myelography to determine the optimal access route to the DRG. The subsequent IG injection consisted of 3 steps. First, a commercially available guide needle was advanced to a position dorsolateral to the DRG, and the dural root sleeve was punctured, leaving the guide needle contiguous with, but not penetrating, the DRG. Second, the custom-made stepped stylet was inserted through the guide needle into the DRG parenchyma. Third, the stepped stylet was replaced by the custom-made stepped needle, which was used for the IG CED. Initial dye injections performed in pig cadavers confirmed the accuracy of DRG targeting under CT guidance. Intraganglionic administration of adeno-associated virus in vivo resulted in a unilateral transduction of the injected DRG, with 33.5% DRG neurons transduced. Transgene expression was also found in the dorsal root entry zones at the corresponding spinal levels. The results thereby confirm the efficacy of CED by the stepped needle and a selectivity of DRG targeting. Imaging-based modeling of the procedure in humans suggests that IG CED may be translatable to the clinical setting.

Key Words • dorsal root ganglia • computed tomography • pain • intraganglionic injection • convection-enhanced delivery • gene therapy • peripheral nerve

Dorsal root ganglia (DRG) contain the first-order neurons of all sensory pathways and are therefore essential structures in pain signaling. Targeted delivery of therapeutic agents into the DRG in animal models is consequently a critical technique to investigate novel analgesic treatments in preclinical pharmacology. In rodents, DRG exposure by open surgical procedure and intra-DRG administration of agents has been demonstrated. While rodent species are used for initial drug discovery and proof-of-concept studies, large animal models are often needed for preclinical toxicity and biodistribution testing of novel drugs. However, no method describing DRG targeting and intra-DRG drug delivery in large animals has been reported.

In the clinical setting, the epidural space adjacent to the DRG is frequently accessed under C-arm fluoroscopy or CT guidance in the performance of transforaminal epidural steroid injections, which are effective in the treatment of radicular pain. This minimally invasive approach may also be occasionally used for radiofrequency modulation of the dorsal, sensory portion of the DRG.

Abbreviations used in this paper: AAV = adeno-associated virus; AAV1 = AAV serotype 1; CED = convection-enhanced delivery; CTF = CT fluoroscopy; DRG = dorsal root ganglia; EGF = enhanced green fluorescent protein; IG = intraganglionic; PBS = phosphate-buffered saline.

This article contains some figures that are displayed in color online but in black-and-white in the print edition.
to treat select pain syndromes. The interventional pain management techniques, however, have not been used to insert the needle tip or to deliver therapeutic agents into the DRG parenchyma.

To optimize the mechanics of intraparenchymal drug administration, convection-enhanced delivery (CED) has been successfully used in the CNS and in the peripheral nerve distal to the DRG. Convection-enhanced delivery uses bulk flow, as opposed to simple diffusion, to enhance distribution of drugs in solid tissues. Studies investigating the needle design needed for efficient CED in the CNS have demonstrated that the stepped needle, consisting of a sharp transition from the wider needle shaft to a narrow tip, improves the volume of distribution of the injectate, allows higher flow rates, and prevents reflux of the injectate along the needle path.

We have developed a CT-guided technique to advance a needle percutaneously into the lumbar DRG in the pig. Successful intraganglionic (IG) drug administration is evidenced by robust transduction of the DRG neurons achieved by CED of adeno-associated virus (AAV). Preliminary clinical translatability of the IG injection is demonstrated by imaging-based modeling of the procedure in humans.

Methods

Animals

Farm pigs of mixed Landrace background (Manthei Hog Farm) weighing between 20 and 30 kg were used. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. The animals were sedated by intramuscular injection of Telazol (tiletamine and zolazepam, 5 mg/kg; Fort Dodge Animal Health), xylazine (2 mg/kg; AnaSed, Akorn), and glycopyrrolate (0.01 mg/kg; Baxter Healthcare). The pigs were then intubated, and general anesthesia was maintained by 1.5%–2% isoflurane (Terrell, Piramal Healthcare).

Imaging

A clinical CT scanner (Definition DS, Siemens Healthcare) with interventional CT fluoroscopy (CTF) hardware and software packages were used. Topograms, preprocedural spiral CT scans, and intraprocedural CTF images were captured using identical acquisition settings as reported previously.

Intraganglionic Injection Assembly

Overview of the Assembly Components. The IG injection was performed using an outer guide needle (22 gauge, 152.7 mm, Quincke tip) and 3 insets (Fig. 1). The insets were passed consecutively through the guide needle in the following order: 1) the proprietary stylet of the guide needle, used to access the intrathecal space adjacent to the DRG under CT guidance; 2) the stepped stylet, used to penetrate the DRG; and 3) the stepped needle, used for IG CED. The guide needle with its first inset (proprietary stylet) was commercially available (Kimberly-Clark). The other 2 insets (the stepped stylet and needle) were custom-made, with design adopted from Krauze et al. and modified for use in the IG paradigm.

Parameters of the Custom Stepped Stylet and Needle. The 175-mm shafts of both the stepped stylet and needle were made of 26-gauge stainless steel tubing (outer diameter 0.01825 in, inner diameter 0.01224 in, wall thickness 0.003 in; Small Parts), and their proximal ends were welded to Luer-style male hubs. For the stylet, solid stainless steel wire (outer diameter 0.009 in, Small Parts) was welded inside the 26-gauge tubing to form a 1.5-mm stepped tip at the distal end of the stylet. For the stepped needle, 5 mm of 32-gauge stainless steel tubing (outer diameter 0.009 in, inner diameter 0.0041 in, wall thickness 0.0025 in; Small Parts) was welded inside the 26-gauge tubing to also form a 1.5-mm stepped tip at its distal end. The diameter and length of both the stepped stylet and needle were selected to fit inside the guide needle while exceeding its length by 2.6 mm at its distal end.

Infusion Apparatus. The male Luer hub of the stepped needle was linked to a male Luer hub of a 100-μl glass injection syringe (Hamilton Co.) by a compression-fitted coupler. The coupler was made of 100 cm of polyethylene tubing (PE/5, Scientific Commodities) and flanked on both sides by nylon female Luer to 1/16-in hose barb adaptors (Cole-Parmer). The infusate was delivered at a controlled flow rate by a syringe pump (Chemex, Stafford).

In Vitro Testing of the Stepped Needle. Efficacy of CED using the stepped needle was compared with that using the guide needle alone in agarose gel. The needle was attached to a stereotactic frame and inserted 5 in (127 mm) into 0.5% wt/vol agarose gel (Life Technologies). Next, 50 μl of 0.4% Evans Blue dye (Sigma-Aldrich) was injected at flow rates between 2 and 20 μl/min. The needle was left in place for an additional 3 minutes after the injection had been completed.

DRG Targeting Under CT Guidance

Planning of the IG Injection Trajectory by CT Myelography. The myelogram was obtained by administration of 0.5-ml diluted contrast medium (300 mgI/ml Omnipaque, Novation) into the dorsal subarachnoid space. The contrast opacified the lumbar thecal sac, including its lateral root sleeves that extend into the intervertebral foramina, and visualized the DRG residing in the lateral intrathecal sleeves. The myelogram therefore allowed determination of the optimal skin entry point, length, and direction of the IG injection path before the IG procedure was initiated (Fig. 2A). Generally, an angle of 60°–70° relative to the sagittal plane was found to facilitate access to the DRG.

Advancement of the Guide Needle to the Lateral Recess of the Intrathecal Space Under CTF Guidance. The guide needle, with its proprietary stylet (first inset) in place, was passed through the skin lateral to the midline and incrementally advanced ventromedially toward the DRG until its tip reached the lateral sleeve of the intrathe-
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Fig. 1. Injection assembly used for DRG targeting and intraganglionic (IG) CED. The 22-gauge 6-in guide needle and 3 insets (proprietary stylet of the guide needle, custom-made stepped stylet, and custom-made stepped needle) were used throughout the 3-step procedure detailed in the main text. A: Overview of the guide needle (with its proprietary stylet in place), the stepped stylet, and the stepped needle. The term “step” refers to the sharp transition between the wide needle/stylet shaft and their narrow tips (arrowhead). B: Longitudinal section of the guide needle with its 3 insets in place: proprietary stylet (upper), custom-made stepped stylet (center), and custom-made stepped needle (lower). C: Cross-section of the stepped needle. G = gauge.
Fig. 2. Targeting the DRG under CT guidance in the pig model. Computed tomography imaging was used to visualize the pertinent spinal anatomy, determine the optimal needle path, and monitor the advancement of the guide needle into the DRG. 

A: Dorsal root ganglia visualization and planning of the injection route. A CT myelogram (left) opacified the thecal sac (arrowhead) and visualized the DRG (arrows). The myelogram was used to determine the optimal trajectory of the needle (line), here shown for the left L-6 DRG. Computed tomography image of the same spinal level without intrathecal contrast is shown for comparison (right). 

B: Placement of the guide needle under CTF guidance. The skin entry point determined by the myelogram was first matched with the corresponding point on the body surface of the animal by placing a radiopaque lead marker (arrowhead) on the skin. The needle was then advanced in increments along its predetermined path (center) until its tip was located directly adjacent to the dorsal surface of the DRG (right). 

C: Confirmation of the guide needle placement. The additional contrast delivered to the left lateral intrathecal sleeve was visualized by CTF as a crescent-shaped hyperdense area (arrowhead), further outlining the targeted DRG. The guide needle on an adjacent slice is not shown to allow better comparison with the myelogram alone presented in panel A. 

D: Bilateral DRG targeting. Once the first needle reached the lateral sleeve of the intrathecal space, a second needle could be advanced to the contralateral DRG using the same technique. The DRG could be safely targeted bilaterally at up to 3 spinal levels during 1 session with no adverse effects. 

E: Needle path and neighboring skeletal structures. Volume-rendered reconstruction (left) provides an overview of the trajectory of the guide needle (thin arrow). The lumbar puncture needle, used for obtaining the myelogram, is also shown (fat arrow). The asterisks indicate the articular processes. Coronal view (center) showing the cauda equina and L-6 DRG (arrows) bilaterally. The tip of the guide needle was passed between the articular processes (asterisks) and into the L5–6 intervertebral foramen. Oblique axial view (right), parallel with the long axis of intervertebral foramen, details the position of the tip of the guide needle immediately dorsal to the DRG (arrow) and ventral to the facet joint (arrowhead). Bars = 2.5 cm.
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cal space at a site directly dorsal to the DRG. Intraproce-
dural CTF imaging monitored advancement of the needle (Fig. 2B), and any deviations from the optimal trajectory were corrected.

Veriﬁcation of the Guide Needle Placement by Contrast Injection. When the needle tip was visualized directly adjacent to the dorsal aspect of the DRG, the stylet of the guide needle was withdrawn, and a small volume (less than 0.1 ml) of the contrast medium was injected. Computed tomography fluoroscopy showed the spread of the contrast medium, within the cerebrospinal fluid surrounding the DRG (Fig. 2C), and veriﬁed that the needle tip had reached the lateral recess of the intrathecal space while not penetrating the DRG itself.

IG Placement of the Stepped Needle. Once the correct position of the guide needle was veriﬁed, the custom-made stepped stylet (second inset) was inserted through the guide needle. The length of the stepped stylet exceeded the length of the guide needle and therefore only the stepped tip of the stylet but not the Quincke tip of the guide needle penetrated the DRG parenchyma. The stepped stylet was then withdrawn and replaced by the stepped needle (third inset). The prior insertion of the stepped stylet prevented clogging of the narrow needle tip.

AAV Preparation

Self-complementary AAV serotype 1 (AAV1) expressing enhanced green ﬂuorescent protein (EGFP) reporter gene under control of the cytomegalovirus promoter/enhancer and the rabbit beta-globin polyA sequence was used. The vector was produced at the Penn Vector Core (University of Pennsylvania).

Detection of AAV Transduction

The animals were euthanized by intravenous injection of pentobarbital. The thoracic aorta and the common iliac arteries were clamped, and the isolated segment was perfused under pressure with 2 L of phosphate-buffered saline (PBS) followed by 2 L of 4% paraformaldehyde in PBS. The harvested tissue samples were viewed for direct EGFP ﬂuorescence by laser scanning microscopy as reported previously. The proportion of the DRG neurons transduced was determined as described by Jacques et al.

Imaging in Humans

To assess possible application to the human DRG, random clinical cases of CT myelography were chosen from the daily imaging schedule for evaluation. Speciﬁcally, cases were sought that had both fat-saturated, gadolinium-enhanced MRI images and a high-quality CT myelogram.

Results

The Stepped Needle Showed Effective CED in Agarose Gel

Comparison of the stepped needle with a regular, nonstepped needle in agarose gel showed superior performance of the stepped needle. Use of the stepped needle re-

sulted in homogeneous distribution of the injectate around the needle tip for flow rates up to 20 µl/min and volumes up to 100 µl (Fig. 3A). In contrast, the nonstepped spinal needle led to tracking of the dye along the needle path.

CT-Guided Injection Accurately Targeted the DRG in Pig Cadaveric Studies

In addition to imaging, correct IG placement of the needle tip was veriﬁed by administration of the Chicago Blue dye in pig cadavers. Figure 3B shows the dye observed in the DRG and spinal roots of the injected spinal levels.

CED Led to a Widespread Distribution of the Injectate in the DRG of Live Pigs

When administered into the DRG in vivo, CED of AAV1 resulted in a robust transduction of the sensory neurons of the injected DRG, with a mean transduction rate of 33.5% (Fig. 3C). Transgene expression was also found in the posterior nerve root and the dorsal root entry zone in the posterior horn of the spinal cord (Fig. 3D), reﬂecting the anatomical pattern of primary sensory neuron transduction previously observed in rodents. Transduction of axons of the anterior nerve root was present and may have been related to the proximity of the anterior nerve root to the DRG and the known ability of AAV1 to transduce both neuronal bodies and axons. An absolute degree of anatomical speciﬁcity was conﬁrmed in terms of the level and laterality of the targeted DRG; there was no transduction of neighboring spinal levels or of DRG on the contralateral side.

Imaging-Based Modeling in Humans Supported Clinical Translatability of the IG Technique

Magnetic resonance imaging of the human lumbar spine identiﬁed the DRG by the presence of gadolinium enhancement (Fig. 4A). The DRG enhances because it lacks a blood-nerve barrier. The other contents of the neural foramen (the nerve roots found proximally and the spinal nerve and its rami found distally) have an intact barrier and therefore show no signs of gadolinium enhancement.

Computed tomography imaging at the corresponding planes showed that there was no skeletal barrier to access the DRG by the posterolateral vector established in the pig model (Fig. 4B). Compared with the pigs, human posterior elements are less bulky, with the laminae and facet joints terminating more medially, facilitating the access to the DRG. Although CT myelography opaciﬁed the lumbar thecal sac, the contrast media did not spread to encompass the DRG, presumably due to the meninges sealing about the nerve roots more proximally in humans than in the pigs. Magnetic resonance imaging was therefore used to provide a physiological cross-reference for the DRG position, which allowed certain identiﬁcation of the DRG tissue on the CT myelogram.

Discussion

Recent development of analgesic therapies directly targeting the primary sensory neurons creates a need for
selective drug delivery into the DRG. An important example explored in the present study is AAV-based gene therapy for pain, which has been found to be efficacious in rodents when the vector was delivered intrathecally. However, subsequent studies in large animals have shown that at least some commonly used AAV subtypes can lead to promiscuous transduction of distant structures, such as spinal cord or brain, which was not observed in rodents. The IG administration markedly reduces this risk associated with intrathecal delivery by minimizing transduction outside the injected DRG. It also allows for a lower therapeutic dose of the virus. The present work demonstrates the efficacy of IG delivery of AAV by CED while achieving the desired anatomical specificity.

In addition to gene therapy, IG injection might provide an important alternative route of delivery for several other novel pharmacological agents that have so far been investigated only in the intrathecal paradigm. Examples include resiniferatoxin and P-saporin, analgesic neurotoxins exerting their therapeutic effect by selective deletions of specific cell populations critical in pain signaling. Delivery of either drug by the IG route might be of future interest and could be tested in the described pig model. In humans, the IG injection is expected to be more straightforward than in the pig model because the human posterior elements tend to be more compact and the intervertebral foramina more readily accessible. Therefore, the IG injection of novel drugs tested in the pig model should be translatable to the clinical setting.

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References

Fig. 4. Modeling the IG injection in humans. Analysis of human spinal anatomy by MRI and CT myelography sustains the feasibility of DRG targeting from a posterolateral approach under CTF guidance in human patients. A: Magnetic resonance images (T1-weighted, fat saturated) of the human lumbar spine, here shown for L-3, L-4, and L-5 levels, identified the DRG by gadolinium enhancement (arrows). B: Computed tomography myelograms of the corresponding segments showed unobstructed access to the DRG. The normodense contours of the DRG (arrows) stood out from the hypodense background of the epidural fat; the intrathecal contrast did not spread into the root sleeves and therefore did not further outline the DRG. The optimal trajectory for accessing the DRG is indicated by a dotted line. Note that the thecal sac is completely opacified with the contrast medium (asterisk) because the human conus medullaris is located at the L1–2 vertebral level. Bars = 5 cm.

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