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Pig lumbar spine anatomy and imaging-guided lateral lumbar puncture: A new large animal model for intrathecal drug delivery

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HIGHLIGHTS

- The development of a novel method for intrathecal drug administration in the pig.
- CT-anatomy demonstrates constraints underlying the failure of human LP techniques.
- A lateral lumbar puncture approach targets the lateral thecal recess.
- This new large animal model offers unique strengths when compared with its alternatives.

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ABSTRACT

Intrathecal (IT) administration is an important route of drug delivery, and its modelling in a large animal species is of critical value. Although domestic swine is the preferred species for preclinical pharmacology, no minimally invasive method has been established to deliver agents into the IT space. While a "blind" lumbar puncture (LP) can sample cerebrospinal fluid (CSF), it is unreliable for drug delivery in pigs. Using computed tomography (CT), we determined the underlying anatomical reasons for this irregularity. The pig spinal cord was visualised terminating at the S2–S3 level. The lumbar region contained only small amounts of CSF found in the lateral recess. Additional anatomical constraints included ossification of the midline ligaments, overlapping lamina with small interlaminar spaces, and a large bulk of epidural adipose tissue. Accommodating the the pig CT anatomy, we developed a lateral LP (LLP) injection technique that employs advanced planning of the needle path and monitoring of the IT injection progress. The key features of the LLP procedure involved choosing a vertebral level without overlapping lamina or spinal ligament ossification, a needle trajectory crossing the midline, and entering the IT space in its lateral recess. Effective IT delivery was validated by the injection of contrast media to obtain a CT myelogram. LLP represents a safe and reliable method to deliver agents to the lumbar pig IT space, which can be implemented in a straightforward way by any laboratory with access to CT equipment. Therefore, LLP is an attractive large animal model for preclinical studies of IT therapies.

1. Introduction

Intrathecal (IT) delivery is an important route of drug administration to bypass the blood brain barrier and directly target the spinal cord. In humans, IT drug administration is routinely achieved using a lumbar puncture (LP). LP can safely be performed at the bedside, does not require specialised equipment, and has been well-established for use in the diagnosis and treatment of many medical problems. Overall, LP is a simple procedure in humans because the spinal cord terminates at the L1–L2 spinal level. The more distal portion of the thecal sac contains only spinal nerves forming the *cauda equina* and a thin continuation of the *conus medullaris* giving rise to the *filum terminale*. In contrast, most other

Abbreviations: IT, intrathecal; CSF, cerebrospinal fluid; CT, computed tomography; CTF, CT-fluoroscopy; LP, lumbar puncture; LLP, lateral lumbar puncture.

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mammals lack this relatively empty space filled with the cerebrospinal fluid and their spinal cord continues distally to a lower lumbar or sacral level (Watson et al., 2008). This anatomical difference between humans and other mammals poses a considerable challenge in modelling LP in an experimental setting.

In view of this anatomical obstacle, two principal approaches to target the IT space were developed in rodents and subsequently translated to larger species. The first technique involves inserting a catheter through the atlantooccipital membrane and then advancing it distally along the subarachnoid space (Malkmus and Yaksh, 2004; Pogatzki et al., 2000; Rijsdijk et al., 2012; Storkson et al., 1996; Yaksh and Rudy, 1976). Although this approach ensures intrathecal placement of the catheter under direct visual control, the advancement of the catheter alongside a major portion of the spinal cord introduces the risk of neurological impairment by damaging the projecting spinal roots. The second technique consists of the direct puncture of the dura mater at a desired spinal level (De la Calle and Paino, 2002; Hylden and Wilcox, 1980; Mestre et al., 1994). This technique allows direct entry into the lumbar subarachnoid space and is analogous to LP in humans. However, the minute size of the intrathecal space in most non-human mammals makes correct needle placement difficult. A shift in the needle position by as little as 0.5 mm can lead to damage of the spinal cord or displacement of the needle tip outside the IT space.

Domestic swine are a large animal species most commonly used in medical research. The size of swine is comparable to humans, and both species share many similarities in their cardiovascular and immune systems. Thus, pigs are a mainstay in a variety of research areas (Fairbairn et al., 2011; McCall et al., 2012; Meurens et al., 2012; Thim, 2010). The pig is an attractive alternative to other frequently used large animal species, such as dogs, cats, or non-human primates, because of their widespread use within the scientific community, relatively low cost, and low risk of disease transmission. Currently, no studies have rigorously examined the porcine spinal anatomy with regard to LP. Although LP is performed on pigs in veterinary practice, the shortcomings described above make this technique unsuitable for research applications that require safe and reliable access to the IT space. Furthermore, IT catheterisation via the cisterna magna is technically difficult in pigs due to a thick muscle layer overlying the cervical spine (McCracken and Kainer, 2006). Consequently, there is no reliable non-invasive technique that allows for the IT delivery of compounds in pigs.

To address the lack of an appropriate animal model, we employed computed tomography (CT) based approach that is used clinically in a variety of neuroradiological spinal interventions, such as LP in patients with spinal deformities, transforaminal or interlaminar epidural injections, and spine biopsy procedures (Wagner et al., 2003). The technological expertise developed in the clinical setting was leveraged in the present study towards the development of a new LP-like technique in the pig. Here, we demonstrate a simple and reliable lateral LP (LLP) technique for access to the pig lumbar IT space under guidance of CT-fluoroscopy (CTF) for IT drug delivery.

2. Materials and methods

2.1. Animals

Male commercial farm swine, of a hybrid Landrace background, weighing between 25 and 55 kg at the time of injection, were used. A total of 15 animals were injected in this study. The pigs were monitored for up to 19 weeks post-operatively. A necropsy was performed after euthanasia. All experiments were carried out in accordance with an approved *Institutional Animal Care and Use Committee* protocol.

2.2. Anaesthesia and perioperative care

General anaesthesia was induced by the intramuscular injection of Telazol (tiletamine and zolazepam, 5 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA), Xylazine (2 mg/kg; AnaSed, Akorn, Decatur, IL), and Glycopyrrolate (0.01 mg/kg; Baxter Healthcare, Deerfield, IL). Anaesthesia was maintained by 1.5–2% isoflurane (Terrel; Piramal Healthcare, Bethlehem, PA) delivered at a rate of 4 L/min with an inspired oxygen fraction of 0.5 using an Ohmeda Modulus 2 anaesthesia machine with an Ohio 7000 ventilator (GE Healthcare, Princeton, NJ). A pressure-controlled ventilation mode was used. The inspiratory pressure was 15 mm H_2O, the respiratory rate was 12 breaths per minute and the expiration-to-inspiration ratio was 2:1. SpO_2, blood pressure, respiratory rate, and body temperature were monitored throughout the procedure.

The animals received a single dose of cefazolin (1 g, intramuscularly; Hospira, Lake Forrest, IL) for antibiotic prophylaxis and carprofen (3 mg/kg; Rimadyl; Pfizer Animal Health, New York, NY) given in one dose subcutaneously prior to the procedure followed by a daily oral dose for three days postoperatively for preventive analgesia. The animals were observed daily following the procedure for neurological deficits and any general signs of distress.

2.3. Imaging

Images were obtained using a clinical CTF scanner (Definition, Siemens Healthcare, Forchheim, Germany) equipped with 3D interventional hardware and software.

Antero-posterior and lateral topograms (low-dose localising radiographs) of the lower abdomen were acquired to obtain a reference for subsequent precise and localised imaging. The tube voltage and current were 120 kV and 35 mA, respectively, with a slice thickness of 0.6 mm. The scanner's standard topogram kernel (T20) was used. The kernel is a scanner-specific parameter that contributes to the spatial resolution and noise texture appearance of the image.

Next, a pre-injection spiral CT scan over the lumbosacral portion of spine was acquired with a radio-opaque guiding grid in place over the spinal column to locate pertinent anatomical structures with respect to the body surface. A tube voltage of 120 kV, rotation time of 0.5 s, collimation of 64 mm × 0.6 mm and pitch of 1.2 was used. Automatic tube current modulation was used to adjust the radiation output for the size of the pig relative to a reference size. Using an image quality reference of 340 mA s resulted in an effective tube current time product (i.e. tube current × time product divided by pitch) of 87–154 effective mA s. A medium kernel (B40) was used to reconstruct images of 3 mm slice thickness.

Next, the scanner was switched to a CTF (interventional) scanning, together with a specialised viewing mode (termed "biopsy mode" by the scanner manufacturer) mode, in which intermittent axial ("step and shoot") scanning were used to monitor the advancement of the needle. The scanner's interventional package allowed for full operation and viewing options from within the scan room through use of an integrated foot switch, a manual controller, and a laser marker illuminating the scanning plane. A set of three adjacent images was produced upon command and then simultaneously displayed. A tube voltage of 120 kV, tube-current, time product of 80 mAs, rotation time of 0.5 s, collimation of 6 mm × 1.2 mm, 2.4 mm slice thickness, and a medium (B40) kernel was used. After the injection was complete, a post-injection spiral CT scan over the same anatomical region was acquired using the identical pre-injection scan/reconstruction settings.

Image post-processing and evaluation were performed on a Syngo MultiModality Workplace (Siemens Healthcare, Forchheim, Germany). Each specific processing protocol is listed in the corresponding figure legend. The viewing window of 400