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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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Abstract

Intrathecal (IT) administration is an important route of drug delivery. Its modeling in a large animal species is a critical step. Although domestic swine is presently a preferred species in preclinical pharmacology, no proven minimally invasive method has been established to deliver agents into the pig 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. The pig spinal cord was visualized terminating at the S2-S3 level. The lumbar region contained only small amounts of CSF visualized in the lateral recesses. Additional anatomical constraints identified were ossification of the midline ligaments; overlapping lamina with small interlaminar spaces; and a large bulk of epidural adipose tissue. Accommodating the pig CT anatomy, we developed an injection technique termed lateral LP (LLP) that employs advance planning of the needle path and monitoring of the IT injection progress. Key features of the LLP procedure were 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 injection of contrast media thereby obtaining 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 and is therefore an attractive large animal model for preclinical studies of IT therapies.

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Keywords

lumbar puncture; pig; swine; computed tomography; intrathecal

1. Introduction

Intrathecal (IT) delivery is an important route of drug administration when bypassing the blood brain barrier or targeting the spinal cord is a main pharmacological objective. IT drug administration can be achieved in humans by lumbar puncture (LP)¹, a routine procedure in the clinical setting. LP can safely be performed at the bedside, does not require specialized equipment, and has been established in diagnosis and treatment for many decades. The procedure is straightforward in humans as the human 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 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 poses a considerable challenge in modeling LP in the experimental setting.

In view of this 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; Rijdsdijk 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 a risk of damage to the projecting spinal roots and of resulting neurological impairment. The second technique consists of 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 allows direct entry into the lumbar subarachnoid space, analogous to LP in humans. However, the minute size of the intrathecal space, which is, unlike in humans, filled with the spinal cord all the way to the sacral level, 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 is one of the large animal species most commonly used in medical research. Not only is its size comparable to humans, but both also species share many similarities in cardiovascular and immune systems. Thus, the pigs are a mainstay in the related research areas (Fairbairn et al., 2011; McCall et al., 2012; Meurens et al., 2012; Thim, 2010;). Ensuing wide experience within the scientific community, together with relatively low cost and low risk of disease transmission, makes the pig an attractive alternative to other frequently used large animal species such as companion animals (dogs and cats) or non-human primates. Up to date, no studies have rigorously examined the porcine spinal anatomy relevant to the LP. Although the LP is performed in veterinary practice in this species, the shortcomings described above make this technique unsuitable in research applications where a safe and validated access to the IT space is required. Furthermore, the IT catheterization via the cisterna magna is technically difficult in pigs due to the thick muscle layer overlying the cervical spine (McCracken et al., 2006). Consequently, there is no rigorous non-invasive technique allowing delivery of agents into the lumbar IT in pigs.

¹**Abbreviations:** IT: intrathecal; CSF: cerebrospinal fluid; CT: computed tomography; CTF: CT-fluoroscopy; LP: lumbar puncture; LLP: lateral lumbar puncture

To address the lack of an appropriate model, we employed a 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 describe how to use the pig as a new large animal model for IT drug delivery demonstrating a straightforward and reliable method to access the pig lumbar IT space under guidance of CT-fluoroscopy (CTF). The method described was termed lateral LP (LLP).

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 followed for up to 19 weeks post-operatively and underwent dissection at the time of sacrifice. All experiments were carried out in accordance with an approved *Institutional Animal Care and Use Committee* protocol.

2.2 Anesthesia and Perioperative Care

General anesthesia was induced by 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) and maintained by 1.5–2% isoflurane (Terrel; Piramal Healthcare, Bethlehem, PA) delivered in 4L/min with inspired oxygen fraction of 0.5. using an Ohmeda Modulus 2 anesthesia machine with the Ohio 7000 ventilator (GE Healthcare, Princeton, NJ). Pressure-controlled ventilation mode, with the inspiratory pressure of 15 mm H₂O, respiratory rate of 12 breaths per minute and expiration-to-inspiration ratio of 2:1. SpO₂, blood pressure, respiratory rate, and body temperature were monitored throughout the procedure.

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

2.3 Imaging

The images were obtained using a clinical CTF scanner (Definition, Siemens Healthcare, Forchheim, Germany) that included 3D interventional hardware and software.

First, antero-posterior and lateral topograms (low-dose localizing radiographs) of the lower abdomen were acquired to determine the extent of a subsequent more precise but anatomically localized 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, selectable parameter contributing to the image spatial resolution and appearance of noise texture.

Subsequently, 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. Tube voltage of 120 kV, rotation time of 0.5 s, collimation of 64 × 0.6 mm and pitch of 1.2 were used. Automatic tube current

modulation adjusted the radiation output for the size of the pig relative to a reference size. Using an image quality reference of 340 mAs resulted in effective tube current time product (i.e., tube-current $\times$ time product divided by pitch) of between about 87 to 154 effective mAs. A medium kernel (B40) was used to reconstruct images of 3 mm slice thickness.

Next, the scanner was switched to the CTF (interventional) mode, in which intermittent axial (“step and shoot”) scanning, together with a specialized viewing mode (termed “biopsy mode” by the scanner manufacturer) 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. Tube voltage of 120 kV, tube-current, time product of 80 mAs, rotation time of 0.5 s, collimation of 6×1.2 mm, 2.4 mm slice thickness, and a medium (B40) kernel were used. After the injection was complete, a post-injection spiral CT scan over the same anatomical region was acquired using the identical scan/reconstruction settings as for the pre-injection spiral scan.

Image post-processing and evaluation were performed on a Syngo MultiModality Workplace (Siemens Healthcare, Forchheim, Germany). The particular processing protocols are listed in the figure legends. The viewing window of 400 Hounsfield units (HU) centered at 40 HU was used unless otherwise specified.

2.4 Intrathecal injection

Animals were placed on the CT couch in the prone position in a radio-lucent cradle with several pads inserted under the abdomen to keep the animal steady and to maintain dorsal convexity of the lumbar spine. A topogram followed by a spiral CT scan over the full lumbosacral portion of the spine was obtained at this time to select a suitable spinal level, with the needle skin entry point and angle determined as detailed below. The overlying skin was then marked, cleaned, and prepared for the procedure in a sterile manner.

Next, the spinal needle (25G 3.5”, Quincke point; Kimberly-Clark, Roswell, GA) was passed through the skin 1–2 cm lateral to the midline followed by gradual advancement ventro-medially at the angle of 10–20 degrees relative to the sagittal plane until the lateral recess of the IT spaces was reached as visualized by the CTF guidance. The IT placement was ascertained by injecting 0.5 ml of iodine containing radio-contrast medium iohexol (300 mgI/ml; Omnipaque; Novation, Chicago, IL) at 1:10 dilution in normal saline. The spread of the contrast media in the cerebrospinal fluid (CSF) was visualized repeatedly to verify the position of the needle tip within the subarachnoid space.

3. Results

3.1 CTF-visualized anatomy of the porcine spine pertinent to the LP

As shown in Fig. 1, we chose a lateral approach through the interlaminar opening, during which the injection needle traversed the following structures in order to reach the IT space: (1) skin; (2) subcutaneous connective tissue; (3) interspinous ligament; (4) *ligamentum flavum*; (5) epidural space; (6) dura mater; (7) potential subdural space; (8) arachnoid membrane; (10) subarachnoid space. The CT scan depicting the anatomical landmarks of the pig spine allowed identification of a number of distinct features compared to human anatomy. These observations were further confirmed during necropsy, which was systematically performed in all animals.

The spinal cord extends to the S2-S3 level in pigs, which differs from adult humans where the spinal cord terminates at the L1–L2 level (Fig. 1A). The principle of performing the LP

safely in humans, which is to insert the needle below the level of the *conus*, can therefore not be implemented in the pig.

The pig epidural space, demarcated by the *ligamentum flavum* dorsally and the dura mater ventrally, was filled with abundant adipose tissue in the pig (Fig. 1B). In humans the fat is usually sparse. The dimensions of the porcine epidural space were therefore much bigger, particularly in larger animals, reaching up to 10 mm in a 50 kg animal. The epidural fat led to anterior bulging of the dura mater with consequent reduction of the IT space size. For a pig in the prone position, this resulted in a diminished amount of CSF in the lumbar area compared to humans. As a result, a spontaneous outflow of the CSF could not serve as an indicator of successful IT injection. In fact, we experienced a lack thereof when the IT space was accessed in the midline in some animals. However, the CT imaging showed that a larger volume of the CSF is accumulated in the lateral recesses of the IT space, which we took advantage of in the injection technique as described in the next section.

Furthermore, the relevant skeletal structures including the spinous processes, vertebral laminae, and articular processes, were bulky as compared to humans (Fig. 1C) in all but young (<35 kg, <3–4 months of age) animals. The space between the spinous processes, as well as the interlaminar space, consequently spanned only few millimeters. In addition, the interspinous ligaments and the *ligamentum flavum* tended to be ossified in older (>35 kg) pigs with the vertebrae (especially in the cervical and lumbar region) being practically fused together. Some intervertebral spaces were therefore not suitable for injection at all. In many others, a direct posterior access in the midline through the ligaments, which is the standard in human LP, was not possible due to the calcification. Moreover, the *panniculus adiposus*, a fat layer separating the skin from the paraspinal muscles, was as thick as 5 cm in the lumbar region and the needle had to be inserted relatively deeply until the underlying structures mentioned above were reached.

These anatomical observations, made evident by the CT and subsequent necropsy, led us to develop an injection technique described below that differs from standard LP in humans.

3.2 Injection technique

An example of a topogram is shown in Fig. 2A and two representative images from the spiral scan are shown in Fig. 1B. These images allowed us to select the most feasible injection site while considering the entire path of the needle in advance. Since we encountered notable inter-individual differences in skeletal anatomy, namely varying dimensions of the interlaminar openings and an uneven degree of ossification of the ligaments, we carefully planned the anticipated injection trajectory including the skin entry point, point of anticipated dura mater penetration, and inclination of the needle to target the lateral recess of the subarachnoid space while avoiding any skeletal or secondarily ossified structures. The needle was therefore inserted lateral to the midline at an antero-medial inclination and advanced towards the lateral recess of the subarachnoid space as documented in Fig. 2B. The lateral modification of the LP technique allowed us to minimize the chance of damage to the spinal cord, to bypass the most frequently ossified central portion of the *ligamentum flavum*, and to take advantage of the local lateral CSF accumulation. We found the L5–L6 segment to be most suitable for injection as the distance between two adjacent vertebral laminae spanned 0.5 cm even in young (25 kg) animals. Although the ligaments tended to be more ossified compared to the upper lumbar levels, this problem was effectively overcome by avoiding the midline structures through needle angulation as described above.

Once the injection site had been selected, the scanner was switched to the CTF mode visualizing the target segment in three adjacent axial images of 2.4 mm thickness (Fig. 2).

Such a setup enabled the operator to view the axial plane as well as to assess the rostro-caudal dimension (the Z-axis, or angulation in the longitudinal plane) during the procedure. The operator was able to acquire images, or to shift the scanned plane rostrally or caudally at any time during the insertion of the needle, allowing monitoring of the needle advancement.

The location of the needle tip was assessed subjectively by the experimenter by passing the needle through two layers of resistance composed of the *ligamentum flavum* and the dura mater (often referred to as a “feel” technique). In some cases this could be correlated correctly with the close proximity of the needle tip to the spinal cord on the CTF imaging. We found, however, that the “feel” technique was often unreliable in pigs as the ossified ligaments gave variable degrees of resistance and as the fat deposits in the epidural space resulted in a relatively large distance between the *ligamentum flavum* and the dura mater compared to the distance between the dura and the posterior surface of the spinal cord. Therefore, the IT placement was verified by a myelogram, where filling of the subarachnoid space by the contrast media and delineating the spinal cord confirmed true subarachnoid placement. Fig. 3 demonstrates the characteristic spread pattern of the contrast within the subarachnoid space.

4. Discussion

The present study demonstrated a pig model of LP for IT drug delivery with a technically verifiable outcome. The CT imaging identified anatomical challenges to performing LP in pigs, which were accommodated by employing a lateral injection technique under CT-guidance, the LLP.

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References

- De la Calle JL, Paino CL. A procedure for direct lumbar puncture in rats. *Brain Res Bull.* 2002; 59:245–50. [PubMed: 12431755]
- Dyce, K.; Sack, W.; Wensing, C. *Textbook of veterinary anatomy.* 3. Saunders; Philadelphia: 2002.
- Fairbairn L, Kapetanovic R, Sester DP, Hume DA. The mononuclear phagocyte system of the pig as a model for understanding human innate immunity and disease. *J Leukoc Biol.* 2011; 89:855–71. [PubMed: 21233410]
- Federici T, Taub JS, Baum GR, Gray SJ, Grieger JC, Matthews KA, Handy CR, Passini MA, Samulski RJ, Boulis NM. Robust spinal motor neuron transduction following intrathecal delivery of AAV9 in pigs. *Gene Ther.* 2012; 19:852–9. [PubMed: 21918551]
- Goodman BS, Bayazitoglu M, Mallempati S, Noble BR, Geffen JF. Dural puncture and subdural injection: a complication of lumbar transforaminal epidural injections. *Pain Physician.* 2007; 10:697–705. [PubMed: 17876368]
- Goransson-Nyberg A, Fredriksson SA, Karlsson B, Lundstrom M, Cassel G. Toxicokinetics of soman in cerebrospinal fluid and blood of anaesthetized pigs. *Arch Toxicol.* 1998; 72:459–67. [PubMed: 9765060]
- Hylden JL, Wilcox GL. Intrathecal morphine in mice: a new technique. *Eur J Pharmacol.* 1980; 67:313–6. [PubMed: 6893963]
- Kern SE, Allen J, Wagstaff J, Shafer SL, Yaksh T. The pharmacokinetics of the conopeptide contulakin-G (CGX-1160) after intrathecal administration: an analysis of data from studies in beagles. *Anesth Analg.* 2007; 104:1514–20. table of contents. [PubMed: 17513651]

- Lee JH, Lee JK, Seo BR, Moon SJ, Kim JH, Kim SH. Spinal cord injury produced by direct damage during cervical transforaminal epidural injection. *Reg Anesth Pain Med*. 2008; 33:377–9. [PubMed: 18675752]
- Malkmus SA, Yaksh TL. Intrathecal catheterization and drug delivery in the rat. *Methods Mol Med*. 2004; 99:109–21. [PubMed: 15131333]
- McCall FC, Telukuntla KS, Karantalis V, Suncion VY, Heldman AW, Mushtaq M, Williams AR, Hare JM. Myocardial infarction and intramyocardial injection models in swine. *Nat Protoc*. 2012; 7:1479–96. [PubMed: 22790084]
- McCracken, T.; Kainer, RTS. *Spurgeon's Color Atlas of Large Animal Anatomy: The Essentials*. 1. Wiley-Blackwell; Ames: 2006.
- Mestre C, Pelissier T, Fialip J, Wilcox G, Eschaliere A. A method to perform direct transcutaneous intrathecal injection in rats. *J Pharmacol Toxicol Methods*. 1994; 32:197–200. [PubMed: 7881133]
- Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdtz V. The pig: a model for human infectious diseases. *Trends Microbiol*. 2012; 20:50–7. [PubMed: 22153753]
- Milants WP, Parizel PM, de Moor J, Tobback IG, De Schepper AM. Epidural and subdural contrast in myelography and CT myelography. *Eur J Radiol*. 1993; 16:147–50. [PubMed: 8462580]
- Pogatzki EM, Zahn PK, Brennan TJ. Lumbar catheterization of the subarachnoid space with a 32-gauge polyurethane catheter in the rat. *Eur J Pain*. 2000; 4:111–3. [PubMed: 10833561]
- Rijdsdijk M, van Wijck AJ, Kalkman CJ, Meulenhoff PC, Grafe MR, Steinauer J, Yaksh TL. Safety assessment and pharmacokinetics of intrathecal methylprednisolone acetate in dogs. *Anesthesiology*. 2012; 116:170–81. [PubMed: 22139590]
- Storkson RV, Kjorsvik A, Tjolsen A, Hole K. Lumbar catheterization of the spinal subarachnoid space in the rat. *J Neurosci Methods*. 1996; 65:167–72. [PubMed: 8740594]
- Suzuki Y, Yeung AC, Ikeno F. The representative porcine model for human cardiovascular disease. *J Biomed Biotechnol*. 2011; 2011:195483. [PubMed: 21253493]
- Thim T. Human-like atherosclerosis in minipigs: a new model for detection and treatment of vulnerable plaques. *Dan Med Bull*. 2010; 57:B4161. [PubMed: 20591344]
- Van Gompel JJ, Bower MR, Worrell GA, Stead M, Meier TR, Goerss SJ, Chang SY, Kim I, Meyer FB, Richard Marsh W, Marsh MP, Lee KH. Swine model for translational research of invasive intracranial monitoring. *Epilepsia*. 2011; 52:e49–53. [PubMed: 21627648]
- Wagner AL. CT fluoroscopy-guided epidural injections: technique and results. *AJNR Am J Neuroradiol*. 2004; 25:1821–3. [PubMed: 15569755]
- Wagner, C.; Wilson, J.; Sabers, S. *Pain Medicine: Fluoroscopic-Guided Interventional Techniques*. Mayo Foundation for Medical Education and Research; Rochester: 2003.
- Watson, C.; Paxinos, G.; Kayalioglu, G. *The Spinal Cord: A Christopher and Dana Reeve Foundation Text and Atlas*. 1. Academic Press; San Diego: 2008.
- Wegner K, Horais KA, Tozier NA, Rathbun ML, Shterman Y, Yaksh TL. Development of a canine nociceptive thermal escape model. *J Neurosci Methods*. 2008; 168:88–97. [PubMed: 18054083]
- Weil L, Frauwirth NH, Amirdelfan K, Grant D, Rosenberg JA. Fluoroscopic analysis of lumbar epidural contrast spread after lumbar interlaminar injection. *Arch Phys Med Rehabil*. 2008; 89:413–6. [PubMed: 18295616]
- Yaksh TL. Analgetic actions of intrathecal opiates in cat and primate. *Brain Res*. 1978; 153:205–10. [PubMed: 98219]
- Yaksh TL, de Kater A, Dean R, Best BM, Miljanich GP. Pharmacokinetic Analysis of Ziconotide (SNX-111), an Intrathecal N-Type Calcium Channel Blocking Analgesic, Delivered by Bolus and Infusion in the Dog. *Neuromodulation*. 2012
- Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. *Physiol Behav*. 1976; 17:1031–6. [PubMed: 14677603]
- Yaksh TL, Tozier N, Horais KA, Malkmus S, Rathbun M, Lafranco L, Eisenach J. Toxicology profile of N-methyl-D-aspartate antagonists delivered by intrathecal infusion in the canine model. *Anesthesiology*. 2008; 108:938–49. [PubMed: 18431131]

Highlights

- Novel method of intrathecal drug administration in the pig
- CT-anatomy demonstrating constraints underlying the failure of human LP techniques
- Approach based on targeting lateral thecal recess termed lateral lumbar puncture
- This new large animal model offers unique strengths compared to its alternatives

Figure 1. Overcoming anatomical constraints in the pig

CT imaging revealed anatomical features in the pig warranting a distinct approach to the lumbar puncture (LP).

A. Multi-planar reconstruction for anatomical landmark demarcation. Three perpendicular planes demonstrate the position of the *conus medullaris* at the S2 vertebral level. Dashed lines show the relative positions of the reconstructed images. (Left, axial; middle, sagittal; right, frontal [tilted forward to be in parallel with the longitudinal axis of the spinal cord in the sacral segment]). CM, *conus medullaris*; EF, epidural fat.

B. Axial imaging used for procedure planning captures two sections at the L6 vertebral level. Top: A section at the level of the intervertebral foramina shows the spinal canal enclosed dorsally by the spinous process and the vertebral lamina. Bottom: A section at the interlaminar space level shows a section suitable for lumbar puncture. The spinal canal is covered dorsally with a visible ligamentum flavum. The hyperdense area in the middle of the ligament represents a calcification that needs to be avoided when inserting the LP needle. The thecal sac contains the spinal cord surrounded by sparse amount of CSF, with somewhat larger volumes being accumulated in the lateral recesses of the thecal sac. The recesses form the lateral aspect of the thecal sac, demarcated medially by the spinal cord and laterally by the epidural space. The optimal injection trajectory is indicated here by a dotted line. The selection of the appropriate skin entry point was aided by placing the guiding grid over the body surface (asterisk). Ca, calcification; EF, epidural fat; TS, thecal sac.

C. A three-dimensional, volume-rendered reconstruction is windowed to the skeletal structures of the lower torso. Distal four lumbar vertebrae transition into the sacrum

consisting of four partially fused units, which is followed by the coccygeal vertebrae forming the tail of the pig. Top: Lateral view. Middle: Postero-anterior view. Bottom: Section at the median plane showing the course and relative dimensions of the spinal canal, demarcated ventrally by the vertebral bodies and dorsally by the spinous processes and vertebral laminae. The injected L5–L6 level was often found to be the most suitable for the injection. The guiding grid (asterisk) marks the location of the body surface. Scale bars: 3 cm.

Figure 2. Execution of the LP under CT guidance

A. A lateral (left) and postero-anterior (right) topogram was acquired prior to the initiation of the procedure (low dose localizing radiograms with a window width of 350 Hounsfield units [HU] centered at 50 HU) with a radio-opaque guiding grid placed over the intended injection area (asterisk). The topogram was used to select an 18–26 cm long spinal segment encompassing distal 2–3 lumbar vertebrae and the proximal portion of the sacrum, which were then imaged using a spiral CT scan. Scale bars: 10 cm.

B. Once the trajectory of the injection had been determined, the scanner was switched into the CTF mode where three contiguous images of 2.4 mm thickness were captured repeatedly throughout the procedure to monitor the needle advancement. In both panels, the angulation of the needle is apparent because the more caudal images (bottom) show the needle tip while the rostral ones show the upper part of the needle shaft only (top).

Left column: The needle was correctly inserted through the L5–L6 interlaminar space according to the trajectory pre-defined in the planning step with the needle tip placed in the right lateral recess of the subarachnoid space. Right column: An example of an unsuccessful needle placement attempt at the same vertebral level. The skin was penetrated at the same point as in the previous image series (left). However, the needle trajectory was incorrect in both planes. The lateral inclination was insufficient to reach the contralateral recess of the subarachnoid space. The excessive caudal angulation resulted in the needle path missing the interlaminar opening (instead contacting the vertebral lamina). In such instances, the needle was partially withdrawn and re-inserted at angulation corrected in both planes.

SA, subarachnoid space; SC, spinal cord. Scale bars: 3 cm.

Figure 3. Verification of the IT placement

The intrathecal placement of the needle tip was confirmed by injecting 0.5 mL of diluted contrast media, iohexol, at the L5–L6 spinal level.

A. The CTF series shows accumulation of the hyperdense contrast in the dependent ventral subarachnoid space with apparent formation of the fluid levels, which distinguishes the correct subarachnoid placement from inadvertent epidural, subdural, or intraparenchymal injection.

B. To illustrate the rostro-caudal distribution of the contrast media, a median sagittal view of the lumbosacral spine was reconstructed from the axial data by multi-planar reformatting. As the animal was placed in the prone position while the contrast was administered, it distributes ventrally along the gravitational gradient, with even caudal and rostral diffusion. Note the small amount of air that inadvertently introduced into the thecal sac during the injection.

Co, contrast media; A, air. Scale bars: 3 cm.