CT-guided injection technique into intervertebral discs in the ovine lumbar spine

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Abstract

Purpose Disc injection to create intervertebral (IVD) disc degeneration (IVDD) has been reported in ovine models, but the techniques have not been thoroughly described. The current ex vivo study aimed to evaluate a computed tomography (CT)-guided injection technique into IVDs in the ovine lumbar spine.

Methods Insertion of needles into the nucleus pulposus was assessed by gross anatomic dissection in two lumbar segments (group A), and injection of liquid within the disc was assessed by discography in six segments (group B).

Results The pathway of the needle was simulated on computer after an initial CT scan, followed by control of the insertion process via a laser beam and monitoring scans. In group A, 20 insertions were assessed and 17 needles (85 %) were successfully positioned in the nucleus pulposus. In group B of 30 injections, the rate of success was 90 %.

Conclusions The current study provides useful clinical information that will help surgeons working with an ovine model for research on IVDD. This model could also be useful to train less experienced surgeons or radiologists to

disc injection. This CT-guided injection seems to offer several advantages such as ease of use, good success rate and safety to important nervous and vascular structures.

Keywords CT · Injection · Lumbar · Intervertebral disc · Sheep

Introduction

Numerous in vivo experimental animal models have been used to study intervertebral disc (IVD) degeneration (IVDD) [1, 2], including minipigs [3–5], dogs [6, 7], goats [8], monkeys [9, 10] and sheep [11–16]. Different approaches have been reported for inducing the disease such as injection and aspiration. The lesions can be induced by injection of either chondroitinase [6, 7, 11] or 5-bromodeoxyuridine [12], puncture [3, 9, 10, 13, 14] or partial nucleotomy [4, 5, 15, 16]. Different approaches to the disc have been reported: invasive [4–8, 13, 15], fluoroscopy [3, 14, 16] or computed tomography (CT) [9, 10, 12] guided.

CT is a modality of choice for guidance in many percutaneous minimal invasive interventional procedures and several CT-guided interventional procedures have been described for management of lumbosacral pain, e.g., periradicular infiltration, percutaneous laser disc decompression, intraarticular steroid injection, percutaneous facet joint denervation and percutaneous lumbar vertebroplasty [17, 18].

In humans, experimental strategies for minimal invasive therapy of IVDD are in the early stages of preclinical development and include intradiscal injection of growth factors, inflammatory inhibitors, proteinase inhibitors, intracellular regulatory substances, genes or cells to

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replenish disc cells and matrix [19]. Those injection techniques might necessitate validation in animal models. CT-guided disc injection has been used to create IVDD in sheep [12]; however, the techniques of injection have not been thoroughly described.

The objective of this study was to design and evaluate a CT-guided injection technique of the IVDs in the ovine lumbar spine, report technical difficulties and measure the rate of successful injection. The current study might provide useful clinical information that will help researchers to induce IVDD in ovine models and test minimal invasive therapies in those models.

Materials and methods

Animals

Ten mature (4–6 years old) Texel ewes killed for reasons not related to lameness or back pain (e.g., mastitis) were used. After evisceration, the lumbar segments were collected with surrounding soft tissues. The segments were clipped, cleaned and frozen at minus 20 °C. Before experimentation, they were thawed to room temperature. All sheep came from the Ovine Research Center of the University of (anonymous). The experimental protocol (KI 10/148) was approved by the local ethical committee for animal welfare.

Injection technique

Two spines ($n = 2$) were used for training and developing the technique. The injection technique is illustrated in

Fig. 1 Reference CT image obtained at the level of the IVD between L2 and L3. 1 Spinous process, 2 m longissimus dorsi, 3 m multifidus dorsi, 4 caudal articular process, 5 cranial articular process, 6 transverse process, 7 vertebral canal with spinal cord, 8 m intertransversarii dorsales, 9 m quadratum lumborum, 10 m psoas major, 11 m psoas minor, 12 annulus fibrosus of the IVD, 13 nucleus pulposus of the IVD

Fig. 1. A 21-G $\times$ 3 $\frac{1}{2}$ in. (0.7 $\times$ 90 mm) needle (Becton-Dickinson, Madrid, Spain) was used.

Identification of the injection plane

The transverse processes of the lumbar vertebra were identified by palpation. A metallic marker (a stainless steel pin) was positioned on the skin longitudinally at the vertical projection of the abaxial extremity of the transverse processes of the lumbar vertebrae corresponding to the targeted IVD (for example, the processes of L2 and L3 if the L2–3 IVD was targeted). The researcher left the room and a CT scan was performed to assess whether the marker was adequately placed (Fig. 2a). One rotation of CT scan was 18 mm long with six slices and covered the whole IVD. In this study, acquisition parameters for monitoring scans were 50 mAs, 130 kV, scan time 0.65 s and slice thickness 1 mm. The slice which best identified the IVD was determined.

Identification of the injection axis

Then, the virtual axis of the needle was drawn on the CT image using software, starting from the metallic landmark and targeting the center of the nucleus pulposus of the IVD. The angle of the axis to the median plane was measured. This angle was used to generate a laser beam from a laser generator at the top of the gantry. The laser beam had an orientation corresponding to the measured angle and was in the plane of the slice which best identified the IVD (Fig. 2b).

Identification of the entry site for the needle

The intersection of the beam and the metallic marker constituted the landmark on the skin to introduce the needle into the skin.

Identification of the injection depth

The direction of the needle was determined by the laser beam. The insertion of the needle consisted of pushing the needle forward while keeping it in the axis of the laser beam (Fig. 2c). The needle was inserted to a distance equal to the distance between the skin and the border of the IVD measured on the initial CT image. Additional CT scans were performed to re-orientate or reposition the needle as necessary. During scans, the clinicians left the room to avoid radiations. When adequately orientated, the needle was advanced into the IVD according to the distance initially measured between the skin and the nucleus pulposus on the CT image. Usually, a “crushing” sensation confirmed penetration of the IVD. The final position was confirmed by CT.

Fig. 2 Injection technique. **a** A CT scan was performed and the pathway of needle was simulated on computer, starting from an entry point (*plain arrow*) vertical to the abaxial extremity of transverse process (*dotted arrow*) and targeting the center of the nucleus pulposus. **b** The angle and the entry point of needle were controlled

by a laser beam created from a laser generator at the top of the gantry. **c** The insertion of the needle consisted in pushing it forward while keeping its base in the axis of the laser beam. Once the tip of the needle was in the nucleus, the contrast medium was injected

Accuracy of the injection technique

The accuracy of the insertion of needles performed by a novice (FN) was assessed in two other spines (group A, $n = 2$). Six other spinal segments (group B, $n = 6$) were used to assess the accuracy of the technique to inject contrast agent into the disc. This was performed by a human radiologist (JFN).

All lumbar spines (group A and B) were scanned beforehand with a view to document CT anatomy. The spines were examined with an Emotion 6 (Siemens, Germany). Transversal reformations were obtained. Acquisition parameters were: 130 mAs, 130 kV, slice thickness 1 mm, rotation time 0.6 s, pitch 0.9. Field of view started from the last thoracic vertebra to the first sacral vertebra. Images were then transferred to a medical digital imaging system (PACS-TELEMIS) for analysis.

In group A, five insertions were performed on each side (the IVDs between L1–L2 and L5–L6; total of 20

insertions). CT scans of each IVD with left and right needles in place were recorded. Each IVD was subsequently dissected by two blinded investigators (JMV, FH) who were not aware of the insertion process. The hypaxial muscles and ventral longitudinal ligament were transected and the IVD was exposed to assess the presence of the tip of the needle in the nucleus pulposus. The insertion was considered successful if the needle penetrated the nucleus pulposus. It was not considered successful if it penetrated the annulus only, missed the IVD or penetrated into the intervertebral foramen. In addition, recorded CT images were analyzed to assess the distance from the thoracolumbar fascia (the distance was not measured from the skin to avoid bias due to variable subcutaneous fat layer and skin folds) to the tip of the needle once it was positioned in the nucleus pulposus. The number of CT scans performed before positioning adequately the needle (as a measure of the difficulty of the technique) was not recorded in this preliminary phase performed by a novice.

In group B, only one side (randomly chosen, either left or right) was punctured on each IVD (L1/L2 to L5/L6, 30 IVDs in total). After accurate positioning of the needle tip was confirmed by CT scan, 0.5 mL contrast agent (Hexabrix, Guerbet, Belgium) was injected into the disc. Images were recorded and assessed blindly by another investigator (JMV). The same parameter as for group A was recorded (distance from the thoraco-lumbar fascia to the tip of the needle). Injection was considered successful only if the contrast was identified within the nucleus pulposus by CT scanning. The cases where needle penetrated the annulus fibrosus only and where the investigator failed to inject contrast (due to the high compactness of the cartilaginous tissue) despite correct placement of the needle were also considered as failure. The number of CT scans performed before positioning adequately the needle (as a measure of the difficulty of the technique) was recorded.

Radiation doses

To evaluate the human body effective dose, the DICOM structured report proposed by the CT (at the end of every procedure) was analyzed. Dose length product in mGy/cm was extracted and computed to human body effective dose in mSv using conversion factors (AAPM report 96).

Results

CT anatomy of the lumbar spine

All spines had six lumbar vertebrae and had a kyphotic conformation. On transverse planes, the vertebral body was triangle shaped in its middle part and transitioned to a circular configuration at the level of the end plates and intervertebral discs. The spinal canal was triangular and large at the level of the intervertebral foramina, but round and smaller elsewhere. The last lumbar vertebra was found cranial to the iliac wings. The transverse processes were directed cranially. The facet joints appeared as “J-shaped” lines (Fig. 1). The surfaces of the caudal (superior) and cranial (inferior) articular processes were, respectively, concave and convex. The joint space was readily identified. Ligaments (supraspinous, interspinous, ventral longitudinal, dorsal longitudinal, flavum) and muscles (m. multifidus dorsi; m. longissimus dorsi; m. psoas major; m. psoas minor, m. quadratus lumborum) were poorly delineated. In all spines, no ilio-lumbar ligament was identified. IVDs were easily identifiable and the nucleus pulposus was more radiotransparent than the annulus, although the outer annulus could not be differentiated from the inner annulus.

Injection technique

In group A, the needles were successfully positioned in the nucleus pulposus of IVDs in 85 % of injections (17 injections on 20). Assessment by gross anatomic dissection and by analysis of final CT scans was similar for all successful cases. Three insertions were classified as failures because one needle penetrated the annulus fibrosus only, another one was directed toward the intervertebral foramen and a third one impacted against the vertebral bone and was bent. Figure 3 illustrates several outcomes of insertions. In group B, the successful rate of injection was 90 % (18 of 20). There were two failures in this group when, though the needle tip was accurately placed and was demonstrated in the nucleus pulposus, no contrast agent could be injected in IVDs due to the compactness of the matrix. In group B, the average number of monitoring scans (those necessary to re-orientate the needle in the insertion process) was 3.75 ± 0.38 times. When insertions were successful in groups A and B, the mean length between the thoraco-lumbar fascia and the tip of the needle was 59.33 ± 1.39 mm. Researchers were never exposed to radiations as they left the room between scans, while the effective dose used was evaluated to be 1.5 mSv.

Discussion

In this study, CT was used to investigate the spinal anatomy of sheep and identify key injection landmarks. Most of our findings were consistent with previous general anatomic descriptions of the lumbar spine and morphologic features of vertebrae [20–22]. In general, sheep spines consist of 7 cervical, 12–14 thoracic, and 6–7 lumbar vertebrae [22]. In this study, six lumbar vertebrae were identified in all spines. In humans, the vertebral antero-posterior (ventro-dorsal) diameter of the vertebral endplates steadily increases from the cervical to the lumbar region, while in sheep it almost stays the same over the whole spine [23]. Another difference between human and ovine lumbar spines is the curvature, which for the sheep is slightly kyphotic rather than lordotic [20, 22]. These features were also visible in all spines in this study. In humans, the last lumbar vertebra is found below the proximal angle of the iliac wings, while it is cranial to the iliac wings in sheep. The transverse processes in humans are smaller than those of sheep. As in man, the caudal (superior) articular facet faced dorso-medially to meet the cranial (inferior) facet. An iliolumbar ligament (between the transverse process of the last lumbar vertebra and iliac crest) was not identified in this series of sheep. It has not been described in the sheep in veterinary literature. In man, strong iliolumbar ligaments check lumbar sacral junction motion [24].

Fig. 3 CT images after insertion of needles. **a** The needle was inserted in the annulus fibrosus. **b** The needle was inserted in a wrong direction toward the intervertebral foramen. **c** The needle bent against the bone or the disc and was deviated ventrally

Ovine IVDs were similar to those observed in humans at CT. All relevant anatomical landmarks for IVDs injection (transverse processes and discs) were easily identified.

The length of the needle used in this study was 90 mm. The average distance required to puncture the IVD was 59.33 ± 1.39 mm. This length was measured from the thoraco-lumbar fascia to the disc. It has been reported that the skin is usually about 2 mm thick and the fat thickness of ovine back may reach 10 mm [25, 26]. So, 90 mm needles should be adequate in most breeds or animals, even in those with more subcutaneous fat.

Injection of contrast was impossible in two cases and necessitated considerable pressure on the syringe in all cases. This might be due to the compactness of the IVD and the viscosity of the contrast medium. However, this was performed in cadavers. Our experience in another series of living animals showed that injection of contrast was much easier.

Different sizes of needles have been used in large animal models for puncture (15 G [9, 10], 16 G [4], 18 G [3],

20 G [9, 10], and 27 G [13]) and injections (16 G [5], 18 G [11, 12], 26 G [6], 27 G [14] and 28 G [7]). Larger needles might facilitate injection of viscous liquids and might be stronger and easier to introduce due to limited bending, thus probably facilitating insertion. However, needle punctures can directly alter mechanical properties via nucleus pulposus depressurization and/or annulus fibrosus damage, depending on the needle size [27, 28]. A review on the effects of localized IVD injury concluded that puncture and stab incisions may also lead to a cascade of biological changes consistent with degeneration, including loss of cellularity, altered biosynthesis and inflammation [29]. The authors also demonstrated that injection of saline with a 25-G needle into a bovine IVD organ culture model induces a loss of cellularity and down-regulation of matrix gene expression. This shows how a minor injury can affect the IVD organ response. The size of needle should therefore be considered in research studies, which should include sham control groups to account for the potential effects of the needle injection [30]. The volume injected is probably also important. For example, a study on rats demonstrated that when the volume injected exceeded a threshold, the discs rapidly exhibited degenerative changes according to radiographic, biochemical and histological analysis [31].

The major difficulty in the current technique was to keep the needle at the axis of the laser beam during initial insertion through the skin. After penetration to deeper tissue, guiding of the needle was easier. A CT scan was performed when the needle was inserted 2–3 cm into muscles, and the needle could be re-orientated if necessary. Despite these technical difficulties, the procedure in this study with CT guidance seemed to be easy as, even operated by a novice (FN, a bachelor student), the success rate of puncture into nucleus pulposus was 85 %. All needles were adequately positioned when the injections were performed by an experienced radiologist (JFN).

This study showed also that CT had the ability to identify and avoid important structures, such as the spinal cord and large vessels (aorta, caudal vena cava). Accurate guidance is particularly important as the characteristic configuration of the vertebral body (small vertebral antero-posterior (ventro-dorsal) diameter in comparison to humans [22] increases the risk of inserting the needle too dorsally or ventrally and damaging the spinal roots or sub-lumbar vessels. Starting the insertion from a point dorsal to the abaxial extremity of the transverse process of the lumbar vertebra seemed important to give to the needle the appropriate direction. This CT technique was useful for all IVDs that were tested. However, we did not assess the injection of the transitional lumbo-sacral disc as this would have required a slice in an oblique cranio-caudal plane which was technically impossible to obtain.

Finally, another advantage was the low radiation dose, both for the patient (1.5 mSv) and the interventional radiologist (as he could stay outside the room during scans).

Conclusion

The current study provides useful clinical information that will help surgeons working with an ovine model for research on IVDD. This model could also be useful to train less experienced surgeons or radiologists in the use of disc injection. This CT-guided injection seems to offer several advantages such as ease of use, good success rate, possibility to avoid important nervous and vascular structures, and low radiation. However, the method developed in this study remains to be validated in large numbers and in living animals.

Conflict of interest None.

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