Development of an in vivo experimental model for percutaneous vertebroplasty in sheep

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Abstract

Introduction: Several studies have described ‘open’ approach techniques for cementation of sheep and goat vertebrae; however, no percutaneous technique has been developed so far for use in non-primates. The aim of this study was to develop an animal model for percutaneous vertebroplasty under clinical conditions.

Methods: In a pilot study with dissected cadaveric ovine vertebrae, the technique and instruments as well as the optimal needle position were determined. In an in vivo animal study using 33 lumbar vertebrae of 11 sheep, a percutaneous vertebroplasty was performed under general anaesthesia. Needle position and cement volume were evaluated from high resolution, quantitative computed tomography imaging.

Results: The percutaneous technique for vertebroplasty was applicable to the vertebral bodies (L1 to L5) of the ovine lumbar spine without any related adverse effects for the animals. The procedure showed a steep learning curve represented by the reduction of the distance between the actual and planned needle positioning (7.2 mm to 3.7 mm; median value) and shorter surgery times (21.3 min to 15.0 min, average) with progression of the study.

Conclusion: The described technique is feasible and repeatable under clinical conditions. This is the first percutaneous vertebroplasty technique for non-primates and we conclude that the sheep is a valid animal model to investigate the effects of cement augmentation in vivo.

Keywords
Animal model, percutaneous vertebroplasty, sheep

Introduction

Percutaneous vertebroplasty and kyphoplasty are minimally invasive techniques for treating osteoporotic fractures and metastatic osteolysis in the thoracic and lumbar spine in humans. These procedures are established, and both are very effective and lead to immediate and lasting pain relief in 80% to 93% of patients (1). Both techniques will be used more and more because demographic data shows that by 2050, the number of people older than 65 years of age will triple, and five to 10% of the population in Europe will be over 80 years old (2). The risk for osteoporotic vertebral fractures increases exponentially with age and the incidence of vertebral fractures for women over 80 years is more than 50% (3).

Since the first reported vertebroplasty from Galibert et al. in 1987 this topic has been investigated in numerous studies (4). Most of them are retrospective studies focusing on the clinical outcome and complications in humans or cadaveric studies (4, 5). There is a surprisingly small number of experimental animal studies and only a handful of live animal models that allow investigation of the in vivo response to the procedure and the bone cement (6–9). Currently, polymethylmethacrylate cement is injected at high viscosity. With a possible extension of the indication for younger patients and the longer life expectancy in the future, there is a demand for resorbable bone cement and efforts are being made to improve the handling properties of the cement.

The reports of animal models that have been published to date all describe open techniques to place the needles into the vertebrae under visualization (6–10). This particular dorso-lateral access is far more invasive and time consuming than the current technique for clinical vertebroplasty in humans. The only percutaneous methods published to date were performed with living primates, however, experimental surgery with primates is expensive and has little ethical acceptance in Europe (11, 12). Additionally, another more recent manuscript of a canine percutaneous model was published (13). The sheep is becoming the animal of choice for testing vertebral implants (14–17). Sheep are readily available and show great homogeneity when selected for age, breed, and sex (18). The size and volume of the vertebrae are comparable to humans, which allow the use of the same implants and instruments as under clinical conditions.
In a comparative in vitro study, the biomechanical behaviour of the ovine lumbar spine was found to be qualitatively similar to that of human specimens (20). Furthermore, the size of the animal, body weight and metabolic rate are similar to humans and substantial efforts are being made to develop a sheep model for osteoporosis (21–25). The bone mineral density of the last four lumbar vertebrae of sheep has been shown from dual-energy X-ray absorptiometry to be in a range from 0.94 to 1.12 g/cm² (25). In comparison, the bone mineral density of human lumbar vertebrae ranges from 0.52 to 0.91 g/cm² (26). The goal of this study was to develop an in vivo ovine model for percutaneous vertebroplasty. The qualitative criteria that were used to evaluate our technique were feasibility, accuracy of needle position in the vertebral body, surgery time and technique related adverse effects.

Methods

The development of the technique presented herein was part of an in vivo animal study, where the effects of vertebroplasty on cardiovascular changes in sheep were investigated (27). All surgeries were performed at the facilities of the Department for Cardiovascular Surgery Research at The University Hospital of Zürich, and were supported by a highly experienced anaesthesiology team. The surgery was performed by adhering to the guidelines for experimental animal surgery, and the local committee (Ethikkommission des Kantons Zürich KEK-No. 204/2007) granted ethical approval.

Prior to the live surgery experiments, the planned positioning of the filling cannula was practiced under fluoroscopic control on an adult sheep cadaver. For the live experiments, a total of 11 skeletally mature mixed breed ewes (3.4 ± 0.9 years old, mean body weight 69.0 kg ± 9.1 kg) were used. Another animal was used as a pilot animal. On each animal, three lumbar vertebrae (L1 to L5) were augmented resulting in a total of 33 samples.

The surgical procedure was as follows: First, the animals were positioned in the dorsal recumbency for cardiovascular instrumentation as required for the main study (27). Details of the instrumentation, anaesthesia procedures applied and the outcomes on cardiovascular alteration due to cement injection are as previously described (27). The animals were then turned and fixed in ventral recumbency on a radiolucent table.

Ovine lumbar vertebrae are extremely narrow in their central aspect (hourglass shaped), being approximately 10 to 20 mm smaller in the frontal plane and 10 to 20 mm longer in cranial-caudal direction as compared to human vertebral bodies. Therefore it seemed appropriate to place one cannula centrally in the cranial half and the second one centrally in the caudal half of the vertebral body.

Although the lumbar facet joints and pedicles of quadrupeds are oriented at an angle of less than 30° to the frontal plane (compared to over 60° in humans) the pedicles are relative short and sagitally oriented (18, 19). To achieve a safe central needle placement, we therefore chose the transition between the transverse process and the pedicle as the ideal entry point, aiming towards the cranial- and caudal hemivertebra respectively, in a 45° orientation to the frontal plane (Fig. 1 and 2).

In order to evaluate the learning curve of the surgeon on the needle placement for the percutaneous technique, the planned needle positions (Q1 and Q2) were defined as follows (Fig. 1): S1 and S2 are the centroids of the cranial and caudal vertebral body endplate surfaces, Q1 is the first quarter point, and Q2 is the third quarter point on the connecting line between S1 and S2.

After making a 1 cm stab incision, a 2.0 mm Kirschner-wire was placed in the dorsolateral cortex from each side in the projection of the pedicles under dorsolateral-fluoroscopic and tactile control. Because the ovine vertebrae have a much harder cortical bone and high bone mineral density compared to osteoporotic humans, it was necessary to prepare the needle path with a 3.2 mm high speed drill. Therefore, a custom made trocar system was slid over the Kirschner-wire and the drill was advanced under fluoroscopic and tactile control. As soon as the medial border of the pedicle was reached, the depth was monitored in the lateral radiography view before

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**Fig. 1** Model for planned needle positioning from computed tomography reconstructions of an ovine lumbar vertebra: divergent para-to transpedicular access to avoid peripheral placement in the narrow centre of the hourglass-shaped vertebra.

**Fig. 2** Intraoperative fluoroscopic monitoring. Demonstration of the placement of the Kirschner-wires in dorsolateral radiography (left) and lateral radiography (right).
advancing the drill to the centre of each half of the vertebral body. A standard 8 gauge filling cannula\(^a\) (4.2 mm outer diameter, 150 mm long) was inserted over the reinserted Kirschner-wire, tightly sealing the pre-drilled canal (Fig. 3).

Under fluoroscopic control, vertebroplasty cement\(^b\) was injected using 1.0 ml syringes from both sides starting at a viscosity of 35 Pa\(\cdot\)s, as measured with a rheometer\(^c\). Cement was injected until extravasates appeared on the fluoroscopic images. The time needed for the needle placement was noted for each vertebrae.

To demonstrate the accuracy of cannula positioning in the series of experiments, the distances (\(d_1\) and \(d_2\)) between the tips of the cannulas (\(K_1\) and \(K_2\)) and their planned localization point (\(Q_1\) and \(Q_2\)) were determined and displayed for each sheep.

To determine the position of each needle tip, the following postoperative procedure was applied. After euthanasia the lumbar spines were explanted and imaged\(^d\) using high resolution computer tomography (123 \(\mu\)m resolution). Computed tomography images were imported into a software programme\(^e\). Semi-automatic segmentation tools were used to associate bone grey values of the images to bone material. As a result of the segmentation process, the image software produced three three-dimensional triangulated surfaces of the outer shell of the vertebral bodies and the cement cloud (Fig. 5). The volume of cement injected into the vertebral bodies was calculated from the computed tomography images taken of the vertebrae as described. The former position of the tips of the filling cannulas, as well as the end of the remaining cementless needle path inside the cement cloud, were determined and marked interactively with image software by setting the landmark points \(K_1\) and \(K_2\).

Using a different software package\(^f\), the cranial and caudal endplates of each polymethylmethacrylate-augmented vertebral body were isolated. The centroids of the surface points were calculated resulting in the two landmark points \(S_1\) and \(S_2\) (Fig. 5). Then the planned positioning of the tips (\(Q_1\) and \(Q_2\)) of the cannulas was calculated. The tips \(Q_1\) and \(Q_2\) corresponded closely to the centres of the cranial and the caudal half of the vertebral body (Fig. 5). With the aid of a Tool Command Language script, the distances between the planned localization of the tips (\(Q_1\) and \(Q_2\)) and the actual position of the filling cannulas (\(K_1\) and \(K_2\)) were calculated.

### Results

The technique of percutaneous vertebroplasty was feasible in all animals of this study. Vertebræ from the first lumbar (\(L_1\)) to \(L_5\) were accessible through a trans- to parapedicular approach. In vertebral caudal to \(L_5\), problems arose due to short vertebral bodies and difficulties in monitoring because the pelvis and limbs of the animals interfered with lateral fluoroscopy. Intravertebral needle placement was achieved in all animals without perforation of the spinal canal; in one animal the ventral cortex was perforated with the plunger. No animal showed adverse reactions during instrumentation, as confirmed by stable cardiovascular parameters including central venous pressure, pulmonary arterial pressure, cardiac output, heart rate, oxygen saturation and arterial blood gas parameters, all of which are described in detail in a related paper (27).

The duration of the procedure to instrument the vertebral bodies with the injection cannulas become shorter over the course of the study. Vertebræ from the first lumbar (\(L_1\)) to \(L_5\) were accessible through a trans- to parapedicular approach. In vertebral caudal to \(L_5\), problems arose due to short vertebral bodies and difficulties in monitoring because the pelvis and limbs of the animals interfered with lateral fluoroscopy. Intravertebral needle placement was achieved in all animals without perforation of the spinal canal; in one animal the ventral cortex was perforated with the plunger. No animal showed adverse reactions during instrumentation, as confirmed by stable cardiovascular parameters including central venous pressure, pulmonary arterial pressure, cardiac output, heart rate, oxygen saturation and arterial blood gas parameters, all of which are described in detail in a related paper (27).

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\(^{a}\) Standard 8 gauge filling cannula: MD Tech, Gainesville, FL, USA

\(^{b}\) Vertebcem: Synthes Inc., Oberdorf, Switzerland

\(^{c}\) RheolabQC Rheometer: Anton Paar, Graz, Austria

\(^{d}\) qCT: Scanco Medical, Brüttesellen, Switzerland

\(^{e}\) AMIRA, Version 4.1: Mercury Computer Systems, Chelmsford, USA

\(^{f}\) Geomagic Studio 9: Geomagic GmbH, Stuttgart, Germany

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course of the surgical procedures. Whereas the instrumentation time was 21.3 minutes on average ($\pm$ 2.3 minutes) for the first sheep, it was 15.0 minutes ($\pm$ 5.0 minutes) for sheep number 5, and 6.7 minutes ($\pm$ 1.2 minutes) for the last sheep. The positioning of the cannula showed a steep learning curve that was represented by the reduction of the distance between the actual and planned needle positioning. Quantitatively, the distances between the planned localization points Q and the position of the tip of the cannulas K decreased with the progression of the study (7.2 mm to 3.7 mm, median values) as represented in Figure 6.

An average of 3.1 ml ($\pm$ 0.9 ml) of polymethylmethacrylate was found within each vertebra as derived from the three-dimensional computed tomography data. As vertebral bodies were maximally filled to evaluate cardiovascular reactions (another part of the study), extravasates were found in all specimens. In all cases, cement extruded through the consistently large central segment vein. In the one vertebra where the ventral cortex was perforated with the plunger, extravasates into adjacent soft tissue occurred early in the procedure. In 19 of the 33 specimens, cement leakage into the spinal canal was observed at the end of the injection; as this was a terminal study set-up, no clinical abnormalities could be documented.

**Discussion**

We developed the first non-primate, large animal model for percutaneous vertebroplasty augmentation. This model has several advantages over the earlier published vertebroplasty models. These advantages are as follows: less invasive access to the vertebral body and as a result a reduction of the access related morbidity, a shorter surgery time, and improved comparability to the clinical setting. Due to the anatomical differences between sheep and humans, the presented model only allows the use of small diameter needles and minimal cement volumes in comparison to the ranges applied for humans (11–13).

Although the anatomy of the facet and pedicle orientation of quadrupeds is different to humans, we were able to use the same instrumentation as used in clinical settings in human patients. Due to the hourglass-shaped ovine vertebrae with sagittally oriented pedicles, the needles had to be inserted through a para- to transpedicular access in divergent directions, which represents a limitation of this animal model since testing of expandable devices may not be possible. In our series, no penetration into the spinal canal through the medial wall occurred. The technique proved to be reproducible and relatively easy to learn, as can be seen from the steep learning curve in surgery time and accuracy of needle placement.

Our other study found that no animal showed any adverse reactions during place-
ment of the cannula. When injecting cement, a temporary and reversible increase in pulmonary artery pressure was noted, as it would be expected in humans (27). Cement leakage is the major complication during vertebroplasty procedures in general, and was observed in all the animals of our study (5). As the effect on cardiovascular alterations due to cement injection was the focus of the cardiac pulmonary study, a maximized cement filling was performed and cement leakage was accepted (27). Unfortunately, the experimental setup did not allow postoperative observations of gait or neurological deficits as the animals were euthanized for further macroscopic and histological investigations. Moreover, due to the presented differences in cement distribution and shape of the vertebrae compared to the human application, the relevance of the animal model for biomechanical investigations is limited.

Due to the required invasive monitoring for the main study, general anaesthesia was mandatory, but percutaneous vertebroplasty intervention might be possible under sedation and local anaesthesia (27).

One limitation of the chosen animal model is the relatively high bone mineral density and the hard cortical bone of ovine lumbar vertebrae, which made it necessary to open the cortex with a high speed drill. Percutaneous drilling includes the risk of iatrogenic injury; the surgeon needs to be familiar with the instruments and some practice is required. The ovariecotomized sheep seems to be a promising model for osteoporosis, however, a post-ovariectomy time period of over 12 months, even combined with low-calcium diets and corticosteroid administration are required (28, 29). If such animals were available, our technique probably could be simplified by omitting the drilling.

Conclusion
The technique presented herein for in vivo percutaneous vertebroplasty in sheep is feasible and repeatable after only a short learning period. This animal model allows investigation of different augmentation techniques in vivo, the testing of new bioactive ceramics and other materials for vertebroplasty and their short- and long-term responses within the bone.

Authors’ contributions
All authors were involved in the study design, and have read and approved the final manuscript. LMB wrote the manuscript and performed all surgery. AG, JK and BA all participated in the experimental surgery; VB participated in the experimental surgery and performed the data- and image processing.

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Conflict of interest
The authors declare that they have no competing interests.

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