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The standardized creation of a lumbar spine vertebral compression fracture in a sheep osteoporosis model induced by ovariectomy, corticosteroid therapy and calcium/phosphorus/vitamin D-deficient diet

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KEYWORDS

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ABSTRACT

Introduction: Vertebral compression fractures (VCFs) are one of the most common injuries in the aging population presenting with an annual incidence of 1.4 million new cases in Europe. Current treatment strategies focus on cement-associated solutions (kyphoplasty/ vertebroplasty techniques). Specific cement-associated problems as leakage, embolism and the adjacent fracture disease are reported adding to open questions like general fracture healing properties of the osteoporotic spine. In order to analyze those queries animal models are of great interest; however, both technical difficulties in the induction of experimental osteoporosis in animal as well as the lack of a standardized fracture model impede current and future in vivo studies. This study introduces a standardized animal model of an osteoporotic VCF type A3.1 that may enable further in-depth analysis of the afore mentioned topics.
Material and Methods: Twenty-four 5-year-old female Merino sheep (mean body weight: 67 kg; range 57-79) were ovariectomized (OP1) and underwent 5.5 months of weekly corticosteroid injections (dexamethasone and dexamethasone-sodium-phosphate), adding to a calcium/phosphorus/vitamin D-deficient diet. Osteoporosis induction was documented by pQCT and micro-CT BMD (bone mineral density) as well as 3D histomorphometric analysis postoperatively of the sheep distal radius and spine. Non osteoporotic sheep served as controls. Induction of a VCF of the second lumbar vertebra was performed via a mini-lumbotomy surgical approach with a standardized manual compression mode (OP2).
Results: PQCT analysis revealed osteoporosis of the distal radius with significantly reduced BMD values (0.19 g/cm³, range 0.13-0.22 vs. 0.27 g/cm³, range 0.23-0.32). Micro-CT documented significant lowering of BMD values for the second lumbar vertebrae (0.11 g/cm³, range 0.10-0.12) in comparison to the control group (0.14 g/cm³, range 0.12-0.17). An incomplete burst fracture type A3.1 was achieved in all cases and resulted in a significant decrease in body angle and vertebral height (KA 4.9°, range: 2-12; SI 4.5%, range: 2-12). With OP1, one minor complication (lesion of small bowel) occurred, while no complications occurred with OP2.
Conclusions: A suitable spinal fracture model for creation of VCFs in osteoporotic sheep was developed. The technique may promote the development of improved surgical solutions for VCF treatment in the experimental and clinical setting.

Introduction

Osteoporosis, a chronic systemic skeletal disease manifested by rarefication of the bone matrix, thinning of trabecular and cortical structures, as well as reduction of osteoid volume, ultimately results in degraded mechanical properties [1,2]. Statistically, about 8% of women over the age of 50 and 27% of women and men older than 65 years will suffer from at least one vertebral compression fracture (VCF) during the remainder of their lifetime [3]. Of interest, recent studies clearly demonstrate an increase in more comminuted fracture types like incomplete burst fractures in the elderly. These fractures occur after minor trauma much rather than during the slow process of creep [4]. Thus, it is of uppermost importance to clarify the nature of the fracture in order to sufficiently plan treatment. Non-operative treatment still serves for minor or less displaced VCFs. In more comminuted

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fractures and severe loss of anterior heights or major destruction and bulging of the posterior wall, there is certain consensus for operative stabilization. Whereas in stable fracture types kyphoplasty or vertebroplasty techniques are indicated, in more comminuted fracture types and incomplete burst fractures, internal fixators or combined fixation techniques (internal fixators plus kypho-/vertebroplasty) are commonly used [4,5]. Unfortunately, all techniques based on cement application have certain drawbacks. One possible disadvantage is the adjacent fracture disease which may be traced back to a high volume cement load adding to the fact that PMMA cement has other biomechanical properties than bone. Adjacent fractures develop in 8% after vertebroplasty and 8 to 26% after kyphoplasty [6-12]. There still remains the problem of cement leakage which accounts for roughly 4-13% of complications in the therapy of VCFs using kyphoplasty (20-70% after vertebroplasty) and presumably increased complication rates for burst fractures [6,13-15]. Furthermore, there is very little known about the healing capacity of osteoporotic spine fractures and how much this is influenced by cement application.

Those specific findings add to osteoporosis associated problems as prolonged healing times and decreased fixation strength of orthopedic implants which are both not solved in clinical practice [16]. There is a need for both experimental and clinical studies focusing on osteoporotic vertebral fractures, the associated healing process and the development of new treatment strategies and implants [16]. Goldhahn et al. [16] were the most recent to claim a lack of specific animal models in studying fracture healing in osteoporosis [16]. However, small animal models, such as frequently used rats, are not suitable because of their size, the absence of the Harversian system and of true lamellar bone; consequently, they lack for trabecular remodeling in the same manner as that of human bone [16-19]. Actually, osteoporosis rarely occurs naturally in animals at all [19]. The sheep's skeleton presents with comparable biomechanical characteristics and comparable sized vertebrae to those of the human skeleton. Multiple studies have demonstrated the sheep's usefulness for osteoporosis research due to their genetic similarity to humans with their estrus cycle, spontaneous ovulation, hormone profiles, and Harversian bone remodeling [17,18,20-22]. Induction of osteoporosis in sheep has been successfully accomplished by local immobilization, e.g., using an external fixator [23], chronic steroid therapy [20,24,25], bilateral ovariectomy [21,24,26], dietary (calcium and vitamin D) restriction [27], combination of the aforementioned [17,18], or central bone regulatory interventions such as intracerebral recombinant leptin application, hypothalamo-pituitary axis disconnection, or pinealectomy [19,28-30]. However, most of these techniques are technically demanding and thus are less suitable for routine application.

In addition, analysis of fracture fixation techniques, healing properties of osteoporotic fractures and the detection of specific failure mechanisms strongly depend on the nature of the fracture. Until now there is no valid fracture model in sheep, able to reproduce a specific fracture type that frequently occurs in humans.

This study presents a slightly facilitated method of osteoporosis induction in sheep. Furthermore, the standardized creation of an osteoporotic incomplete burst fracture type A3.1 was introduced. It was hypothesized that (1) ovariectomy, weekly corticosteroid therapy, and a calcium/phosphorus/vitamin D-deficient diet in a sheep model could induce severe osteoporosis of the lumbar spine after 5.5 months of treatment; (2) these osteoporotic effects can be effectively proven via pQCT and micro-CT analysis; and (3) creation of lumbar VCFs type A3.1 in standardized manner using a mini-lumbotomy is achievable.

Ethics statement

The study was conducted in strict accordance with the European Union legislation for the protection of animals used for scientific purposes; therefore, it was approved by the state's Animal Ethics Committee (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern, Rostock, Germany; permit no. 7221.3-1.1-007/13).

Material and methods

Animal preparation and osteoporosis induction

Twenty-four 5-year-old female Merino sheep (mean body weight: 67 ± 1 kg; range: 57-79) were ovariectomized (OVX) bilaterally (OP1) using a left side flank incision and general anesthesia according to the procedure of Mohamadnia et al. [31]. OVX was performed under general anesthesia induced by 2.0 mg/kg body weight ketamine (Pfeizer GmbH, Germany), 0.5 mg/kg body weight midazolam (Roche Pharma AG, Germany), and 0.010 mg/kg body weight fentanyl (Albrecht GmbH, Germany) intravenously (IV), and maintained with 1.0-1.5% isoflurane (Baxter AG, Germany) and a FiO2 of 0.4 after 24 hours of fasting. Preoperatively, 0.1 mg/kg body weight xylazine was injected intramuscularly (IM) for sedation. Intra- and post-operatively, extreme effort was made to minimize suffering via the administration of perioperative analgesia with 2.5 g metamizole (MSD Animal Health GmbH, Germany) IV for the second half of the procedure and 0.005-0.010 mg/kg body weight fentanyl IV if the duration of the procedure exceeded 1 hour. The postoperative analgesia regimen was administered according to the 2010 guidelines of the Society of Laboratory and Animal Science [32] and included a combination of 25-50 mg/kg body weight metamizole IV/IM q6h (later administered orally) and 0.005-0.010 mg/kg body weight fentanyl IV on demand. An IV prophylactic antibiotic (cefuroxime, Fresenius Kabi AG, Germany) was administered intraoperatively and postoperatively; 1 g menbutone (Boehringer Ingelheim Vetmedica, Germany) IM was administered to promote absorption.

Further osteoporosis induction was performed according to Zarinklam et al. [18]. The animals were housed in a shed protected from sunlight without litter. Each sheep received a pelleted mixed feed (720 g/d, Table 1) in addition to straw (550 g/d). In contrast to the other nutrients the daily calcium, phosphorus, and vitamin D_3 intake was in deficit to the recommendations [33-35] and calculated with 1.6 vs. 5.0 g, 2.6 vs.4.0 g, and 183 vs. 448 IU, respectively. From postoperative day 7 onward a weekly injection (IM) of 1.3 mg/kg body weight dexamethasone and 0.028 ml/kg body weight dexamethasone-sodium-phosphate were administered. Steroid administration was continued for 4.5 months and gradually reduced during the following 4 weeks to prevent any withdrawal symptoms.

Table 1
Composition and constituents of the pelleted mixed feed for sheep; calcium/phosphorus/vitamin D-deficient based on the nutrition recommendations for a 70kg body weight sheep.

Composition (in %)		Ingredients (in % of DM)	
Barley	51.00	Dry matter (in %)	87.94
Maize	20.00	Crude protein	14.02
Rye	15.00	Utilizable crude protein (in g)	132.82
Premix[1]	5.50	Crude fat	2.89
Wheat bran	5.00	Crude fiber	4.03
Molasses	2.00	Crude ash	2.49
Urea	1.50	Calcium	0.07
		Phosphorus	0.32
		ME (MJ/kg)[2]	10.6

[1] per kg diet: vitamin A 12.000 IE, vitamin D_3, 250 IE, vitamin E 90 mg, manganese (oxide) 50 mg, zinc (oxide) 50mg, calcium iodate 0.5 mg, sodium selenite 0.15 mg, cobalt carbonate 0.20 mg;
[2] Metabolisable Energy.