RESEARCH ARTICLE

Evaluation of BP-ONJ in osteopenic and healthy sheep: comparing ZTE-MRI with μ CT

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Objectives: Bisphosphonate-associated osteonecrosis of the jaw (BP-ONJ) is a side effect of antiresorptive treatment that is increasingly prescribed for patients with osteoporosis or malignant diseases with bone metastases. Surgical treatment of BP-ONJ requires adequate pre-operative imaging. To date, CT is the imaging standard in clinical routine; however, defining the extent of the pathological area is difficult and soft tissues are poorly displayed. MRI with zero echo time (ZTE-MRI) to display hard tissues enables a precise display of calcified structures and soft tissues for the delineation of bone necrosis and soft-tissue reactions.

Methods: BP-ONJ was induced in eight sheep by extraction of two premolars in the left mandible and zoledronate (ZOL) administration. Eight sheep without ZOL administration served as the control group. Four sheep of each main group underwent osteopenia induction *via* ovariectomy, glucocorticoid administration and a calcium-free diet. After sacrifice, the area of tooth extraction was harvested and scanned with micro-CT (μ CT) and ZTE-MRI. Two trained dentists analyzed digital imaging and communications in medicine data sets using three-dimensional imaging software. The periosteal reaction and the remaining extraction sockets were measured.

Results: BP-ONJ was evident, and the remaining extraction sockets were observed in all animals treated with ZOL. Periosteal reactions were more pronounced in animals treated with ZOL, and they appeared broader in ZTE-MRI.

Conclusions: BP-ONJ lesions in the sheep mandible can be detected using μ CT and ZTE-MRI. Although illustration of sequester was more consistent using the μ CT, ZTE-MRI was advantageous in evaluation of periosteal reaction.

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Introduction

Bisphosphonate-associated osteonecrosis of the jaw (BP-ONJ) is a side effect of antiresorptive treatment.

BP-ONJ was firstly described in 2003 in relation to bisphosphonate treatment [zoledronate (ZOL) and pamidronate] of bone metastases. Its growing prevalence is due to an increase of the prescription of anti-resorptive medication in different indications including osteoporosis and myeloma. Regarding a growing number of patients who develop similar bone necrosis after treatment with the receptor activator of nuclear

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factor kappa-B ligand antibody Denosumab and several cases of bone necrosis in patients receiving tyrosin kinase inhibitors, the hypernymph medication-related osteonecrosis of the jaw has been proposed.¹

Even though most lesions occur after invasive dental procedures such as tooth extractions, the exact pathomechanism is not elucidated yet. In patients with progressive periodontal lesions, exposed bone can be seen before tooth removal. Also, non-exposed variants of the condition that can be visualized using single-photon emission tomography with low dose single slice CT or MRI have been published.^{2,3} As bisphosphonates are mainly enriched in the bone that underlies remodeling and the remodeling rates in the alveolar processes seem to be higher than in other bones, it is most likely that the local concentration of bisphosphonates in the alveolar processes is elevated.^{4,5} High levels of bisphosphonates have been shown to lead to apoptosis of osteoclasts.⁶ Given that especially in bones with a high turnover, a certain level of osteoclastic activity is necessary to maintain the tissue's integrity, a lack of osteoclasts leads to osteonecrosis. Otto *et al*⁷ describe an interaction of different factors: an oral infection or dentoalveolar surgery may lower the local pH value of the bone and lead to release and activation of toxic levels of bisphosphonates; following which the influences on osteoclasts (suppressed bone remodeling), immune cells (specific infection), angiogenesis (ischaemia) and mucosa (soft-tissue toxicity) result in BP-ONJ.

Osteolytic lesions with cortical bone destruction, osteolysis, sequestrum formation and sclerotic regions with periosteal bone proliferation are typical for BP-ONJ.^{8–12} Surgical procedures include the resection of necrosis in advanced stages (American Associations of Oral and Maxillofacial Surgeons; AAOMS) and the early surgical approach and safe wound closure.¹³ Surgical procedures require precise pre-operative imaging to define necrotic areas and delineate resection margins.

Conventional two-dimensional radiography does not deliver information on the spatial dimensions of necrotic areas. However, panoramic radiography together with three-dimensional CT is the most frequently used imaging techniques in clinical routine.¹⁴ Other imaging modalities for the detection of BP-ONJ are multislice CT,¹⁵ CBCT^{10,11,16} and MRI.^{12,15–17} Hybrid single-photon emission tomography with low-dose single-slice CT and scintigraphy enables detection of BP-ONJ in pre-clinical stages;³ however, scintigraphy has a low spatial resolution and does not allow for metric analysis of lesions.¹²

MRI is a non-ionizing method with superior soft-tissue contrast compared with radiographic techniques. However, in conventional MRI with T_1 and T_2 weighted contrast, BP-ONJ lesions show a variable signal.¹⁸ Areas of exposed bone are associated with low signal in T_1 and T_2 weighted images, whereas soft tissues with concomitant oedema and inflammation cause a high-signal T_2 contrast.¹⁵ Compact bone and teeth are as well associated with a low signal due to fast signal decay and are poorly displayed. By reducing the time

between signal excitation and detection to a few microseconds, MRI with zero echo time (ZTE-MRI),¹⁹ it was possible to depict dentin, cementum and enamel of extracted teeth with a voxel volume of $150 \mu\text{m}^3$ using small-bore animal MR systems at 7.0 T and 9.4 T.^{20,21} Therefore, ZTE-MRI was chosen as an imaging tool for the detection of soft- and hard-tissue alterations in BP-ONJ.

Hitherto, numerous groups have used small animal models to study the pathogenesis of BP-ONJ. However, because rodents have distinct differences in bone architecture and biology, large animal models are needed. Sheep have been proposed and used as animal models for *in vivo* studies of bone metabolism. The induction of osteopenia with morphological changes of the spine and jawbone has been documented.^{22,23} The occurrence of BP-ONJ after tooth extraction and spontaneous exposition of bone was demonstrated in healthy and osteopenic ewes under ZOL administration.²⁴ The protocol for induction of osteopenia in sheep suggested by Veigel *et al*²³ was adopted in this study.

The aim of the study was to display morphological changes in sheep with BP-ONJ using ZTE-MRI and micro-CT (μ CT) and to compare the diagnostic abilities of both imaging methods with regard to hard- and soft-tissue alterations. The hypothesis was that with both imaging modalities, the morphological changes were displayed in the same way. Control groups without osteopenia and with bisphosphonate administration as well as with osteopenia and without bisphosphonate administration were established.

Methods and materials

Animal model

All procedures within the study were carried out in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility according to the Swiss laws of animal welfare and were approved by the local animal welfare commission of the official veterinary authorities (authorization number 2012_10).

16 adult female Swiss mountain sheep were randomly divided into 2 groups of equal size (Group 1 and Group 2). Inclusion criteria were adulthood, weight between 55 and 70 kg and general health, assessed using clinical and blood examinations. All animals had a 2-week acclimatization period prior to the experimental phase. The weight of the animals was assessed at entry and on a weekly basis during the experimental phase. During post-operative care, an everyday score for behaviour, breathing, feeding, temperature and wound healing was measured. Animals were fed a maintenance ratio of hay and had water *ad libitum*.

In Group 1, eight animals underwent ovariectomy, followed by 16 weeks of glucocorticoid induction with weekly injections of 54 mg of dexamethasone and 1.5 ml of dexamethasone sodium phosphate and a low calcium

Figure 2 Coronal cross-section of a specimen scanned using micro-CT with anatomical and pathological structures. a, remaining extraction socket; b, periosteal reaction; c, bone marrow; and d, cortical bone.

Measurements of periosteal reaction on the buccal and oral aspect of the alveolar bone were correlated with Bland–Altman plots for the two examiners (E1 and E2) and for the two imaging modalities MRI and μ CT. The arithmetic mean of the differences is stated with 95% CI. The oral and vestibular measurements were considered as dependent variables and were therefore evaluated separately. The interclass correlation coefficient with 95% CI was calculated for the measurements of each examiner at the different time points (t1, t2 and t3).

Results

Clinical findings

In all animals treated with ZOL (Groups 1a and 2a), BP-ONJ with exposed and necrotic bone, infection and purulent drainage was observed. In the control groups, the extraction sites healed uneventfully.

Imaging findings

The extraction sites in osteopenic and healthy sheep without bisphosphonate medication (Groups 1b and 2b) showed a complete bony regeneration and an intact cortical bone layer after regeneration. Radiography and MRI of extraction sockets in sheep treated with ZOL showed remaining extraction sockets and a periosteal reaction. In osteopenic sheep without BP-ONJ, a periosteal reaction was observed.

The interclass correlation coefficient calculated for repeated measurements and for the two examiners

showed values between 0.972 (CI 0.93–0.991) and 0.996 (CI 0.99–0.999) for MRI and between 0.929 (CI 0.758–0.989) and 0.997 (CI 0.988–1) showing a high agreement between examiners and time points of measurement.

The estimated marginal means of the repeated measurements by two examiners were calculated for the comparison of specimens. In Group 1b, only MRI measurements were available. In μ CT cross-sections, the periosteal reaction was not assessable because it was not visible. In Group 2b, the periosteal reaction was not detectable in all μ CT images of four specimens and on MR images of one specimen. All estimated marginal means are stated in Table 1.

Periosteal reaction was not significantly different between Groups 1a and 2a, as well as Groups 1b and 2b. However, there was a significant difference between the periosteal reaction in Groups 2a and 2b. The difference of periosteal reaction in Groups 1a and 1b was only detected on the vestibular aspect but not on the oral aspect.

The difference between mean measurements in MRI and μ CT images was 0.59 mm (CI 0.46–0.72 mm) on the vestibular aspect and 0.32 mm (CI 0.21–0.43 mm) on the oral aspect. The measurements for periosteal thickness in μ CT were lower than in MR images (Figure 5a,b).

The difference of the mean measurements made by E1 and E2 were 0.05 mm (CI $\times$ 0.03–0.13 mm) for the oral aspect in MRI and 0.03 mm (CI $-$ 0.07–0.13 mm) for the vestibular aspect in MRI. For μ CT measurements, the difference was $-$ 0.07 mm (CI $-$ 0.16–0.01 mm) for vestibular measurements and $-$ 0.05 mm (CI $-$ 0.16–0.06 mm) oral measurements (Figure 5).

Figure 3 Coronal cross-section scanned using zero echo time MRI. a, remaining extraction socket; b, periosteal reaction; c, bone marrow; and d, cortical bone.

Table 1 Estimated marginal means and confidence intervals on vestibular (vest) and oral aspects in Groups 1a, 1b, 2a and 2b stated in millimeters

Image	Group 1a		Group 1b		Group 2a		Group 2b	
	vest	oral	vest	oral	vest	oral	vest	oral
MRI	2.37 (1.69 to 3.04)	1.58 (0.48 to 2.67)	0.4 (−0.15 to 0.95)	0.42 (−0.48 to 1.3)	2.36 (1.89 to 2.84)	1.96 (1.19 to 2.74)	0.33 (−0.22 to 0.88)	0.26 (0.63 to 1.16)
Micro-CT	1.7 (0.38 to 2.96)	1.23 (−0.36 to 2.82)	–	–	1.83 (0.92 to 2.74)	1.65 (0.53 to 2.77)	–	–

Discussion

The present study used μ CT and ZTE-MRI to quantify and compare morphological alterations in corresponding cross-sections of identical specimens with BP-ONJ. μ CT was previously described as an adequate technique to display morphological changes of the jawbone;²³ however, it is limited by a poor soft-tissue contrast. ZTE-MRI was successfully used by Weiger *et al*²⁵ to depict the trabecular microstructure of bone after dehydration and removal of soft tissue. This study examines the display of hard and soft tissues with ZTE-MRI and μ CT.

BP-ONJ was successfully induced in sheep treated with ZOL, and the clinical picture of BP-ONJ could be correlated with the image of non-mineralized extraction sockets in μ CT and MRI. These findings are in accordance with Farias *et al*⁹ and Bisdas *et al*,¹⁸ who described remaining extraction sockets among other radiological findings such as destruction of cancellous bone, sequestration, erosion of cortical bone in their patient collectives. The specimens were segmented in μ CT with the help of gray values to detect other morphological changes than the remaining extraction sockets. Owing to the small specimen size with a focus on the extraction socket, sequestra formation and osteolysis could not be outlined to their full extent. Therefore, a quantification of these findings was not possible.

ZTE-MRI and μ CT data displayed a reaction of the periosteum around tooth extraction sites in osteopenic sheep after the administration of bisphosphonates. To the knowledge of the authors, this is the first study that quantified and compared the periosteal reaction in BP-ONJ between two imaging modalities.

The periosteal reaction is characterized by the deposition of newly formed bone on the cortical bone surface in response to local insult or generalized processes. Therefore, it is only visible in CT after the mineralization of the newly formed bone.²⁶

Previously, several authors^{8,11,18} detected periosteal reactions with CT, characterized by new periosteal bone formation but did not quantify their findings. The number of patients with periosteal reactions was relatively low in relation to the study collective.⁸ The infrequent documentation of periosteal reaction could be due to the effect that only mineralized parts of the newly formed bone were visible.²⁶ In a patient collective with late stages of the disease, a higher number of periosteal reactions was documented.¹⁸

The findings of this study confirm differences in the visibility of periosteal reactions between μ CT and ZTE-MRI. A periosteal reaction was not visible in μ CT and MRI cross-sections of all specimens. In the control groups, a periosteal reaction could not be assessed in seven specimens in μ CT and in one specimen in MRI, respectively. The periosteal reaction measured in μ CT had a minimum width of 0.5 mm. Therefore, the extent

Figure 4 Alignment of the specimen scanned within an individual co-ordinate system. The x-axis is oriented axial to the mandible at the most caudal point of the mandible, the y-plane is oriented sagittal to the mandible and located in the centre of the extraction socket (vertical lines). The periosteal reaction is measured in the coronal cross-sections of micro-CT (left) and MRI (right) with a distance of 3 mm between each measuring plane (horizontal lines).

Figure 5 Bland–Altman plots of the difference between the mean MRI and mean micro-CT (μ CT) measurements on the vestibular and oral aspects. Each dot represents a measurement of one examiner at one time point (overall two examiners at each three time points). SD, standard deviation; ZTE-MRI, zero echo time MRI.

of periosteal bone formation might have been underestimated and less often detected in μ CT compared with MRI. The threshold for detection of alterations was not regarded as a result of the spatial image resolution and voxel size, respectively, as it was higher for μ CT (30 μ m) than was for MRI (117 μ m). However, ZTE-MRI was not only able to display hard-tissue but also soft-tissue alterations such as reactive periosteal hypertrophy and newly formed bone in mineralized and non-mineralized stages.

Previous studies on imaging of BP-ONJ used CBCT,^{10,16} CT,^{8,15,18} positron emission tomography CT¹⁶ and MRI^{12,15–18} and focused on the display of osteolytic areas, osteosclerosis, sequestra formation and erosion of the cortical bone.^{8,9,11,12,16} As a result, the extent of morphological changes in BP-ONJ may not be reliably assessed using the current imaging methods.^{8,15} The heterogeneity of imaging protocols, the differing stages of the disease and the individual and subjective assessment protocols in the mentioned studies do not allow a general conclusion for the radiographic visibility of BP-ONJ.

BP-ONJ in osteopenic and healthy sheep, resulted in comparable periosteal reactions. In healthy sheep with and without BP-ONJ, a significant difference of the periosteal reaction was observed. In osteopenic sheep with and without BP-ONJ, a statistical difference of the periosteal reaction was only calculated for the vestibular aspects of the specimens. This effect was observed despite deviating means and could be due to the small sample size in Group 1a that was caused by an early loss of two animals with major infections after osteopenia induction using ovariectomy and glucocorticoid treatment.

In all healthy and osteopenic sheep without ZOL administration, uneventful wound healing was observed. However, a periosteal reaction was detected in osteopenic sheep. Induced osteopenia followed by periosteal thickening is in accordance with Bissere

et al,²⁶ who suggested periosteal apposition as a compensation for endocortical bone loss in pre-menopausal females. Even though the periosteal reaction in all specimens was studied in μ CT and ZTE-MRI, a limitation of this study is the missing histomorphometric evaluation. Therefore, the actual value of periosteal bone formation could not be assessed. Nonetheless, a quantitative evaluation of the periosteal reaction was possible with identical specimens examined using both imaging modalities. Another limitation is that specimens were scanned after sacrificing of the animals which is not directly transferable to the clinical setting in patients. However, with the additional information that is supplied with contrast agents, the use of ZTE-MRI may be even more advantageous. The clinical use of ZTE-MRI described by Weiger et al²⁷ gives a perspective for the use of this imaging modality for *in vivo* examination of BP-ONJ. Further studies should focus on different stages of the disease to determine the visibility of early pathological alterations.

Conclusions

A periosteal reaction was observed in ZTE-MRI and μ CT in all sheep diagnosed with BP-ONJ. A broader and more frequent periosteal reaction was measured in ZTE-MRI compared with μ CT. Therefore, ZTE-MRI proved a reliable imaging modality to display hard- and soft-tissue structures of the mandible and might be applied for the display of earlier stages of BP-ONJ.

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