Can we induce osteoporosis in animals comparable to the human situation?

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ABSTRACT

Osteoporosis is a chronic systemic bone disease of growing relevance due to the on-going demographic change. Since the underlying regulatory mechanisms of this critical illness are still not fully understood and treatment options are not satisfactorily resolved, there is still a great need for osteoporosis research in general and animal models in particular.

Ovariectomized rodents are standard animal models for postmenopausal osteoporosis and highly attractive due to the possibility to specifically modify their genetic background. However, some aspects can only be addressed in large animal models; such as metaphyseal fracture healing and advancement of orthopedic implants. Among other large animal models sheep in particular have been proven invaluable for osteoporosis research in this context.

In conclusion, today we are able to influence the bone metabolism in animals causing a more or less pronounced systemic bone loss and structural deterioration comparable to the situation found in patients suffering from osteoporosis. However, there is no perfect model for osteoporosis, but a variety of models appropriate for answering specific questions. Though, the appropriateness of an animal model is not only defined in regard to the similarity to human physiology and the disease itself, but also in regard to acquisition, housing requirements, handling, costs, and particularly ethical concerns and animal welfare.

Introduction

Osteoporosis is a chronic disease characterized by systemic deterioration of bone mass and microarchitecture leading to skeletal fragility associated with an increased risk of fractures. This socially and economically dramatic health problem of the developed world is going to be even more critical as a result of the on-going demographic change [1,2]. Chrischilles et al. calculated that every second white woman above 50 years of age would suffer from an osteoporotic fracture during her lifetime – leading to disability, increased mortality, and financial burden [3]. Ross et al. reported that also every third man would suffer from osteoporotic fractures during his lifetime [4]. Today in Europe not only 22 million women but also 5.6 million men suffer from osteoporosis and the calculated health burden is within the range of other widespread chronic diseases [5].

Till today the underlying regulatory mechanisms of bone metabolism leading to progressive loss of bone mass and structural integrity are not fully understood and surgical as well as non-surgical treatment options are yet not satisfactorily resolved. This is why massive efforts are underway to further investigate this critical illness.

With this review article we aimed at giving an overlook of some established animal models for osteoporosis focusing on important general characteristics of suitable models. Furthermore, ethical concerns changed dramatically in society and research community during the past decades, which is why there is nowadays a need for a much more critical view on all established animal models.

General comments

For the on-going osteoporosis research animal models are of great value and still essential at this time. But "if a disease or condition is not fully understood, how can one design a good animal model of the disease? This is the "*animal model paradox*" [6]. Not surprisingly, there is up-to-date no ideal animal model for osteoporosis – and probably never will be – because osteoporosis is not a single disease but a family of disorders negatively affecting the human bone turnover and animals are despite all similarities in bone structure and metabolism obviously not humans. For this reason, every model struggles with specific pro and cons and can only be able to mimic certain aspects of the human disease. So the question remains, whether we can induce osteoporosis in animals comparable to the human situation?

In vitro analyses of different bone cell types are extremely helpful in answering important questions at the molecular biological level, in particular questions regarding intra- and intercellular signaling. Furthermore, these studies are able to

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reduce the amount of animal experiments needed. However, these experiments can never address the highly relevant interactions of various organ systems, or structural and biomechanical issues in complex organisms. In addition, the American Food and Drug Administration (FDA) recommends ovariectomized animals as the preferred model for bone loss research [7] and due to the guidelines of the World Health Organization (WHO), drug effects must be demonstrated in appropriate animal models for osteoporosis [8]. But appropriateness in this context has different dimensions! Thus the specific animal model not only has to be appropriate in terms of imitating the human disease, but also when looking at costs and availability as well as ethical concerns. Reinwald and Burr defined concrete parameters that should be looked at when choosing a large animal model for osteoporosis, such as 1) appropriateness as a model of estrogen deficiency (i.e., significant bone loss induced by estrogen depletion), 2) specific biological and physiological characteristics (e.g., osteonal bone remodeling), 3) cost and availability, 4) housing/ spatial requirements, 5) manageability during an experiment, 6) reproducible results, 7) minimal ethical/societal implications, and 8) predictive of skeletal effects of potential osteoporosis therapies in adult humans [9].

Small animal models for osteoporosis

Small animal models – namely rodents – are well established as models for osteoporosis. The ovariectomized mouse and rat are up-to-date standard animal models for postmenopausal bone loss [10–12]. In contrast to large animals, experiments with small animals are less costly and time consuming, requirements for housing and handling are of smaller dimensions, and ethical implications are in general lower in comparison to large animals. In addition, the possibility to specifically modify the genetic background of mice, made these animals extremely attractive for studying bone metabolism and disorders [13]. The genetic modification of single genes gives the great opportunity to identify the role of specific factors, membrane proteins, signaling pathways, or else. For example, our group could recently demonstrate the importance of the transmembrane receptor *Kremen-2* (*Krm2*) in the regulation of bone formation in a knock-out mouse model (Fig. 1) [14]. Recently it succeeded to alter also the genetic background of rats, making them again more attractive as models for bone loss [15].

However, beside all these advantages of rodents, there are issues that can only be addressed in large animal models; such as metaphyseal fracture healing – the 'hot-spot' area of osteoporotic fractures [2] and advancement of orthopedic implants comparable to those used in humans [16,17]. In addition, repeated histomorphometric analyses, substantial blood and urine samples, as well as iliac crest biopsies can only be performed in large animals [18] (Fig. 2).

Large animal models for osteoporosis

Searching for an appropriate animal model for postmenopausal osteoporosis, it has to be considered that spontaneous menopause is only found in humans, Old World monkeys and great apes. Since most other mammalian species experience lifelong estrous cycles [9,19] bone-loss caused by estrogen deficiency cannot be observed naturally in these animals [18]. Furthermore, in all quadrupeds the static and biomechanical loads – especially of the extremities and spine – is different to those in humans [9,20].

In international literature different species are described as large animal models for bone loss, such as sheep, goats, dogs, pigs, and non-human primates. The latter obviously do show the most similarities to human bone structure and metabolism
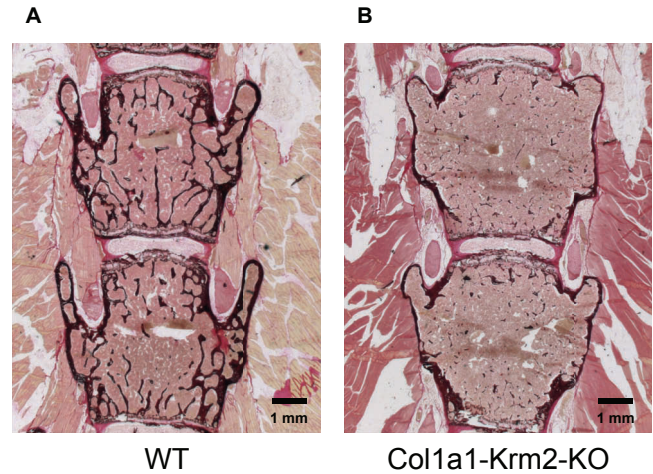
Fig. 1. Images of von Kossa/van Gieson staining of non-decalcified vertebral body sections from (A) 10 weeks old female wildtype mice and (B) *Col1A1-Krm2*-transgenic mice. The osteoblast-specific over-expression of *Kremen-2 (Krm2)* in transgenic mice results in severe osteoporosis indicating the regulatory role of *Kremen-2* in bone remodeling [14].

from all animal models available. However, ethical concerns and legal restrictions are highest in these animals. Disadvantageous is furthermore, that experiments with non-human primates are very cost intensive and only legalized in very few centers around the world [21–23]. This gives reason why these animals – although very close to human physiology – are not appropriate as standard models for osteoporosis.

Beside non-human primates pigs do have many characteristics of bone structure and metabolism in common with human. Additionally, their gastrointestinal system as well as the water and electrolyte homeostasis is close to human [24–26]. The similarities between both species are best documented through the fact that organs of pigs are used for Xeno-transplantations in humans [27]. Disadvantageous however is the fact, that adult domestic pigs weight up to 200 kg and especially male subjects tend to be aggressive. This critical combination makes it sometimes impossible to do further experiments or even to take blood samples without performing general anesthesia in these animals [25]. However, mini-pigs might be an attractive alternative to work with [28]. Recently genetic modifications in pigs via e.g. nucleus-transfer-technology were successfully performed, such as establishing inducible RANK-Ligand overexpression systems as models for inducible systemic bone loss [29,30].

Beagle dogs have also been characterized as models for human bone loss [31]. They do also show bone structure and metabolism comparable to humans with cortical and trabecular bone remodeled by bone multicellular units (BMUs) [32]. However, the data published about the effects of ovariectomy on bone structure and turnover are inconsistently and the effects vary significantly between different anatomical sites [33–36]. In addition, ethical issues, especially in the societies of the western hemisphere, are highly relevant using dog models, thus this model is also not appropriate as a standard model of bone loss.

Sheep in particular, have proven invaluable in orthopedic research [9,37,38] and should be therefore discussed in more detail on the following pages.

The Ewe

Female sheep (ewes) are well established as model animals in orthopedic research. Some of the advantages of sheep are: their docile compliant nature [18], their simple husbandry needs, low costs of acquisition and maintenance, and availability of aged
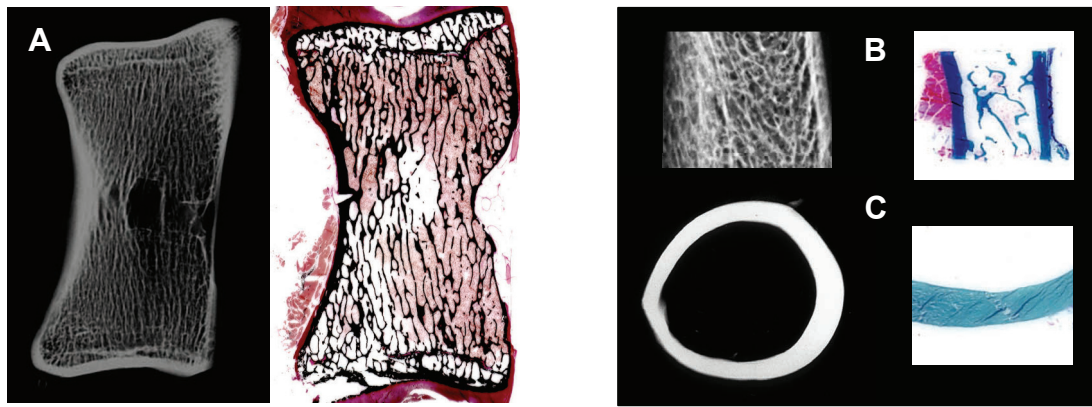
Fig. 2. Different bone biopsies from (A) Lumbar spine, (B) Iliac crest, and (C) Femoral cortex of a 6-year-old ewe. Each figure shows an overview of the contact radiography (left) and the corresponding histological image (right).

(>6 years) animals in large numbers [6,39]. In addition, ethical and societal implications are generally less sensitive compared to other large animal models [9].

The macro- and microarchitecture of sheep bone is comparable to human bone; trabecular and cortical bone with Haversian systems, as well as bone remodeling performed by bone multicellular units (BMUs) is found in both species [6,9,40] (Fig. 3). The cortical bone of young sheep is plexiform. Although older sheep (~1 year) show already bone remodeling with well-developed Haversian systems [6,18,39,41,42], remodeling of all primary osteonal bone is not observed until 7–9 years of age [9,37]. In addition, the relevance of biochemical bone turnover markers such as alkaline phosphatase, osteocalcin or crosslinks could also be demonstrated in sheep [40,43]. Beside the described similarities in bone structure and metabolism between human and sheep, there are some differences that have to be taken into account. Thus the bone mineral density (BMD) and bone mineral content (BMC) are significantly higher in sheep compared to humans (BMD lumbar spine (mg/cm³): human ~180 vs. sheep ~440, BMC (mg): human ~80 vs. sheep ~240) leading to an even more pronounced increase in mechanical stability (fracture stress (N/mm²): human ~1.2 vs. sheep ~13.2) [32]. These might explain why even ewes with marked bone-loss still show relatively high BMD and BMC values and osteoporotic fractures barely ever occur in these animals. Since BMD and bone turnover parameters change significantly in sheep throughout the year [40,41,44], appropriate control groups are essential and experiments should – whenever possible – span all four seasons to minimize these effects [18].

Sheep are predominantly polyestrous/seasonal short day breeders and therefore sensitivity of bone metabolism on estrogen deficiency varies with the season [45]. In addition, cycle characteristics vary significantly between human and sheep (e.g. cycle length (days): woman 28 vs. ewe 17; approximate estrogen peak (pg/ml): woman 300–600 vs. ewe 8–10) [9,46]. The less pronounced influence of estrogen on bone turnover in sheep might explain the minor effects on bone mass or structure after ovariectomy in these animals (see below).

A major disadvantage of herbivores/ruminants as animal models is the different gastrointestinal system. These animals are therefore obviously not suitable for studying effects of orally administered drugs [9,18].

Ewe models for osteoporosis

There are several sheep studies published focusing for example on fracture healing [47,48], orthopedic and dental implants [49,50], bone substitutes [51–55], as well as anti-
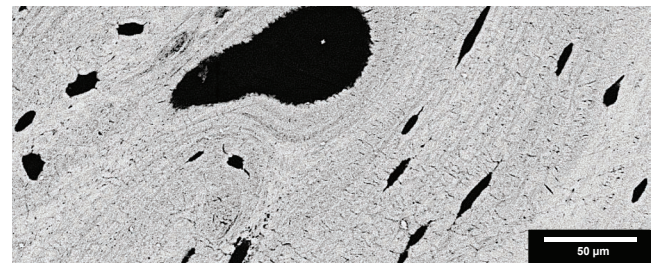
Fig. 3. Electron microscopic image of the femoral cortex of a 6-year-old ewe showing lamellar bone and well developed Haversian systems comparable to the situation found in adult human.

osteoporotic drugs [56,57]. Whereas in the past predominantly aged, ovariectomized ewes or glucocorticoid sheep have been used as models, in the last decade sheep models for central bone regulation were additionally introduced [58–60].

Models for postmenopausal osteoporosis – ovariectomy

The ovariectomy (OVX) in sheep is a simple and safe surgical approach. Although BMD reduction is reported three and six months after OVX [61,62], long-term studies questioned the sustainability of the described bone loss. Several groups demonstrated that the BMD levels and bone turnover markers stabilized and returned to pre-OVX levels around six months after OVX [59,62–64]. The observed rebound effect is explained by histomorphometric analyses showing that the increase in bone resorption is compensated by a simultaneous increase in bone formation [59]. However, other groups reported significant changes of bone mass and micro-structural parameters, as well as biomechanical properties 12 and 24 months after OVX [65–69]. Whereas other studies failed to show significant changes 12 months after OVX [70]. Nevertheless, the relevance of hormonal influences on bone metabolism in sheep is supported by data from studies showing that estrogen and selective estrogen receptor modulators (SERM's) are able to significantly increase bone mass in these animals [57,71].

Taken together, the influence of estrogen on bone metabolism in sheep seems to be comparable to those found in humans. However, significant changes in bone mass and/or structural parameters are due to distinct compensation mechanisms difficult to predict.

Models for steroid-induced osteoporosis

Glucocorticoid therapy in sheep leads to significant-to-dramatic bone loss, structural deterioration and biomechanical
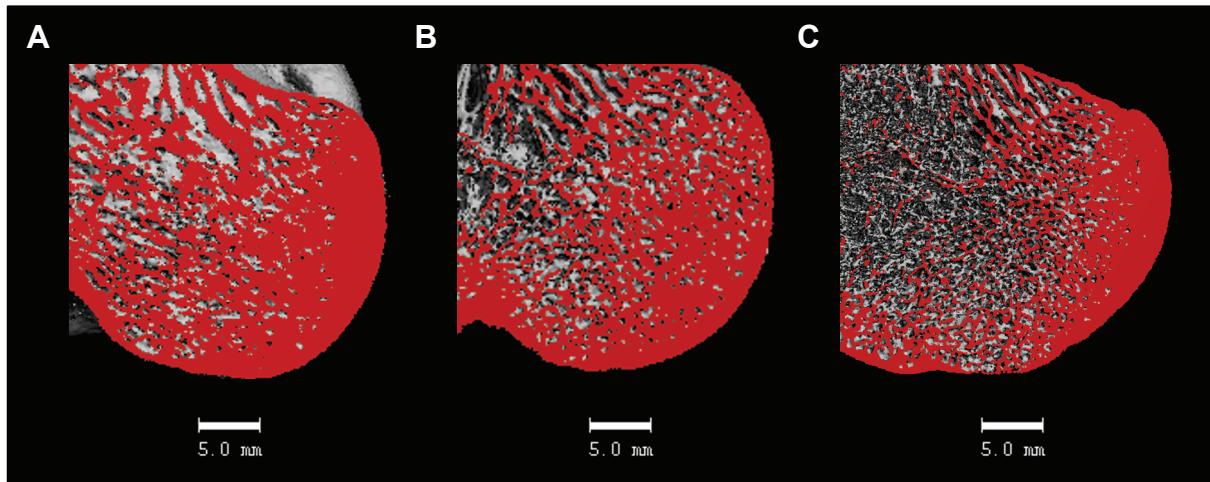
Fig. 4. The surgical hypothalamo-pituitary disconnection (HPD) leads to significant bone loss of both cortical and trabecular bone in sheep; indicating the important role of central regulatory mechanisms of bone mass and structure in mammalians. Frontal view of high-resolution peripheral quantitative computed tomography (HR-pQCT; XtremeCT®, Scanco Medical, Switzerland) of the medial femur condyle of (A) untreated control ewe, (B) 12 months, and (C) 24 months post HPD procedure.

impairment comparable to the conditions found in steroid-treated humans [43,72]. Advantages of this approach are the ease and reliability of induction of pronounced cortical and trabecular bone loss [73,74]. Therefore, this model is the most widely used sheep model for systemic bone loss/osteoporosis. However, major disadvantages of this treatment regime are the need for continued glucocorticoid injections to achieve the bone loss and the compromised animal welfare with documented severe side effects, such as massive infections and hair-loss [43,73–75]. These side effects can be reduced by decreasing the number of glucocorticoid administrations (using equal total amounts) without reducing the impact on bone metabolism [76]. Nevertheless, ethical implications limit the value of this model as systemic side effects preclude studying skeletal physiology in bone loss situations. Furthermore, ethical implications in terms of severe side effects "cannot be overlooked" [77] in bone loss models based on polypragmatic treatment regimes, such as combination of OVX + glucocorticoid therapy + diet restriction + movement restriction [75,78], which is why these models are nowadays obsolete [38].

Models for centrally induced bone loss

Experiments in rodents gave us insides in central bone regulation and helped to identify Leptin as a potential candidate for regulation of this superordinate controlling system of bone metabolism [79–83]. With the intracerebroventricular (ICV) application of recombinant Leptin in ewe leading to significant decline in bone formation and bone mass, it could be demonstrated that this system is also important for bone regulation in large remodeling animals [59,84]. However, this model is not appropriate as a regular model for studying osteoporosis, due to the very high running costs (recombinant Leptin) and the very complex neurosurgical procedure and setting necessary for implementation [38].

Subsequently, our group implemented a ewe model for centrally induced bone loss by surgical disconnection of the hypothalamo-pituitary axis (HPD) [60]. This neurosurgical approach is sufficient to implement a profound bone loss that affects cortical as well as trabecular bone. Thereby the bone loss developed continuously over time and was sustained without any further treatment (Fig. 4), which is important in reducing running costs, as well as improving animal welfare. Histomorphometric analyses could identify a pronounced low

turnover situation with simultaneously depressed osteoblast and osteoclast function as reason for the observed bone loss. However, surgical disconnection of the pituitary gland from the hypothalamus leads obviously to several systemic alterations as a result of blood level changes of different hormones (LH, FSH, T3, T4, IGF-1, cortisol, and leptin) [60]. These systemic changes need to be addressed when interpreting results generated in this model [38,85].

Another ewe model for central bone regulation described by Egermann et al. is based on melatonin deficiency caused by surgical pinealectomy [58]. Melatonin is not only secreted centrally by the pineal gland but also in bone marrow cells [86] and has a significant influence on osteoblast proliferation, differentiation and activity [87–89] as well as on bone mass and structure [90]. Significant reduction of bone mass is described in this model after 6 and 30 months post pinealectomy in comparison to control. Although the reported bone loss was limited, the decrease reached the level of significance and no other treatment was necessary beside the pinealectomy [58].

Conclusion

Osteoporosis is a chronic systemic bone disease of growing relevance due to the on-going demographic change. Since the underlying regulatory mechanisms of this critical illness are still not fully understood and treatment options are not satisfactorily resolved, there is still a great need for osteoporosis research. For this research animal models are still essential and also recommended from the American Food and Drug Administration (FDA) as well as the World Health Organization (WHO).

But can we induce osteoporosis in animals comparable to the human situation? Looking at osteoporosis as a disease causing a systemic bone loss – the answer is 'Yes!' However, this is very simplistic and not helpful in studying underlying regulatory mechanism of the disease itself. Furthermore osteoporosis is not one single disease but a family of disorders negatively affecting the human bone turnover and structure. That gives reason why there can never be just one or the perfect model! Every model struggles with specific pro and cons and can by its nature only be able to mimic certain aspects of the human disease. But this means vice versa, that even animal models representing only some aspects of the respective human condition/disease may be useful [91].

Ovariectomized rodents (mouse and rat) are up-to-date standard animal models for postmenopausal osteoporosis.

Furthermore, the possibility to specifically modify the genetic background of these animals gives the great opportunity to identify the role of specific gene products in bone metabolism and/or bone disease. However, some aspects can only be addressed in large animal models; such as metaphyseal fracture healing and advancement of orthopedic implants.

Among other large animal models sheep in particular have been proven invaluable for osteoporosis research. Beside the similarities in bone structure, metabolism and hormonal regulation, sheep have simple husbandry needs, a compliant nature, are available in large numbers, costs for acquisition and maintenance are in general low, and societal and ethical implications are low compared to other large animal models. However, study results always have to be interpreted against the background of strain, age, season, diet, skeletal site and hormone-cycle characteristics – therefore, appropriate control groups are crucial.

The ovariectomized ewe is an established model for post-menopausal osteoporosis due to the well-documented hormonal influence on bone metabolism in sheep. However, the unreliable rebound effect after OVX and the only minor impact on bone mass questioning this model suitable as a standard model for human osteoporosis. In contrast, Glucocorticoid treatment has a major impact on bone turnover in sheep and leads to conditions comparable to those found in steroid-treated humans. However, adverse side effects cause unacceptable discomfort and illness of the experimental animals and questioning this model – without substantial improvements of the animal welfare – as ethically acceptable. Last but not least, animal models of centrally induced bone loss are doubtless very complex systems. However, these models might be useful for studying central regulatory mechanisms of bone metabolism as well as testing new implants and/or bone substitutes and/or anti-osteoporotic drugs. The HPD model for example might be attractive for studying effects of anti-osteoporotic drugs in a pronounced low-turnover situation comparable to the situation found in patients suffering from senile osteoporosis [38].

In conclusion, we are able to influence the bone metabolism in animals causing a more or less pronounced systemic bone loss and structural deterioration comparable to the situation found in patients suffering from osteoporosis. However, there is not THE model for osteoporosis nor a perfect model, but a variety of models appropriate for answering specific questions. However, the appropriateness of an animal model for osteoporosis is not only defined in regard to the similarity to human physiology and the disease itself, but also in regard to acquisition, housing requirements, handling, costs, as well as ethical concerns and animal welfare. This implies that for specific questions many different aspects have to take into account – not only the impact on bone mass and structure caused by a therapeutic intervention.

Conflict of interest

The authors have no conflict of interest.

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