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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].

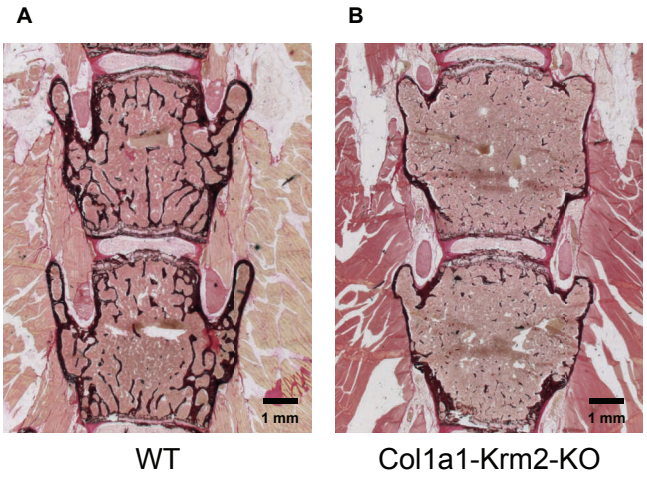

Fig. 1. Images of von Kossa/van Gieson staining of non-decalcified vertebral body sections from (A) 10 weeks old female wildtype mice and (B) *Col1A1-Krm2*-transgenic mice. The osteoblast-specific over-expression of *Kremen-2 (Krm2)* in transgenic mice results in severe osteoporosis indicating the regulatory role of *Kremen-2* in bone remodeling [14].

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 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