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Improvement of large animal model for studying osteoporosis

Zdzisław Kiełbowicz[1], Anita Piątek[1], Janusz Bieżyński[1], Piotr Skrzypczak[1], Ewa Chmielewska[2], Paweł Kafarski[2], Jan Kuryszko[3]

[1]Department of Surgery, Faculty of Veterinary Medicine,
University of Environmental and Life Sciences in Wroclaw, 50-366 Wroclaw, Poland
[2]Department of Bioorganic Chemistry, Faculty of Chemistry,
Wroclaw University of Technology, 50-370 Wroclaw, Poland
[3]Department of Biostructure and Animal Physiology, Faculty of Veterinary Medicine,
University of Environmental and Life Sciences in Wroclaw, 50-375 Wroclaw, Poland
anita.piatek@up.wroc.pl

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Abstract

The aim of the study was to determine the impact of steroidal medications on the structure and mechanical properties of supporting tissues of sheep under experimentally-induced osteoporosis. A total of 21 sheep were used, divided into three groups: a negative control (KN) (n = 3), a positive control (KP) (n = 3) with ovariectomy, and a steroidal group (KS) (n = 15) with ovariectomy and glucocorticosteroids. All animals were kept on a low protein and mineral diet and had limited physical activity and access to sunlight. Quantitative computed tomography was the examination method. The declines in the examined parameter values in the KS group were more than three times higher than in the KN group. The study suggests that a glucocorticosteroidal therapy accelerates and intensifies processes taking place in the course of osteoporosis. The combination of glucocorticosteroids with ovariectomy, a restrictive diet, limited physical activity, and no access to sunlight leads to a decrease in radiological bone density.

Keywords: sheep, osteoporosis, large animal model, glicocorticosteroids.

Introduction

In spite of being thoroughly studied, osteoporosis has still been considerably problematic in humans. It is the most common disease of bone that leads to increased bone fragility. The main features of this disease are primarily a decrease in bone mass and abnormal bone microarchitecture. A characteristic feature of bone is its lifelong reconstruction, where bone resorption (osteoresorption) is followed by a process of bone renewal (osteogenesis). These processes are closely involved in ensuring homeostasis. Glucocorticoid drugs are frequently responsible for secondary osteoporosis because they speed up and enhance the degree of changes in the course of osteoporosis, thus increasing the risk of pathologic fractures to a very large extent.

The influx of information on osteoporosis is proportional to the importance it plays in modern civilisation. An indicator of the achievements of civilisation, and especially of medicine, is the extension of human life. Osteoporosis develops insidiously, unnoticed for many years until the occurrence of fractures. Understanding the factors that contribute to its development, methods of early detection and the possibility of prevention and treatment are therefore necessary to reduce the scale and effects of the disease. Osteoporosis is a disease incidence of which increases with age, and the problems grow commensurately with the prolongation of life. Over 25% of women and 10% of men over 60 years of age experience osteoporosis with all its consequences. Epidemiological studies indicate further that the cause of an increase in the number of osteoporotic fractures is not only the extension of life, but also the changes in lifestyle and eating habits. In most cases, patients have primary osteoporosis, not associated with other disorders. It affects older people of both sexes over 70 years of age, and some women of 50 years of age. In this second group, it is connected with a decrease in the secretory

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activity of the ovaries. These organs produce female sex hormones with oestrogens among them, and they along with others have a protective effect on bone tissue. Approximately 5% of patients have secondary osteoporosis. This means that the loss of bone mass is a result of abnormal secretion of hormones caused by the disease.

Due to the increasing threat posed by osteoporosis, there is a need to search for new and better drugs that inhibit its development. It has become necessary to find an animal model which will allow further progress in work to control the disease. The animal should have a suitable size large enough for collection of multiple samples for testing and performing experimental procedures on. Moreover, the construction and structure of the bone and metabolic changes should be similar to human. Progress in medicine is possible in large part thanks to the experiments conducted on mammals. Osteoporosis progresses gradually, requiring many years of ongoing research in order to verify whether the treatment is effective. The results appear very slowly and it takes a long time to collect test results. A major obstacle to research is the diversity in human lifestyles, and unequal impact of health-related factors associated with osteoporosis, such as nicotine, alcohol, and diet, upon osteopathic patients. These factors constitute a major hindrance when research requires a homogeneous study group, because they influence the results (11, 24). Trauma associated with falls poses the most serious human health risk associated with the disease, resulting in prolonged hospitalisation and an increased number of deaths (9). The animal model provides more uniform experimental material and a chance for a comprehensive study of potential therapies. A properly chosen animal model for the study of osteoporosis minimises the limitations of research which would otherwise pertain were research performed on human. Moreover, the high cost and long duration of clinical trials are other reasons for bestowing the key role in the study of osteoporosis (10) upon the animal model. However, choosing the right animal as a model for studying osteoporosis is not easy, due to the need to consider a number of factors. This is very well expressed by Rodgers *et al.* (22): "convenience, relevance (comparability to the human condition) and appropriateness (a complex of other factors that make a given species the best for studying the particular phenomenon)". Considering the animal model for osteoporosis, the following must be taken into account: age at peak bone mass, the presence of any correlation between age and bone loss, the occurrence of reversible oestrogen-dependent bone loss, the appearance of spontaneous fractures, and whether changes occur both in cancellous bone (trabecular) and cortical. In addition, it should be adjudged whether one animal model is sufficient, or different models are needed for various degrees of osteoporosis. As research progressed, it became apparent that the animal model used for the study of osteoporosis passes the test of some, but not all of the above considerations. A suitable animal model for experiments should be an analogue giving access to information, and what is more it should be characterised by genetic uniformity. In selecting an appropriate model, the canon of knowledge about its biological properties, availability, and husbandry costs should be taken into account. The feasibility of standardising specific results, the adaptability of the species to research activities, the environmental aspects, and ethical and social consequences (5) must likewise not be omitted from contemplation.

Many species of animals were studied. Primates have many advantages compared to other animal models for studies of osteoporosis. Their internal organs and systems, such as the gastrointestinal tract, endocrine system, and bone metabolism, are the closest to human. The results of the loss of cancellous bone described by Miller *et al.* (17) and Mann *et al.* (16) show the similarity between human and primates in the response of the skeletal system to the inhibition of ovarian function. In primates menopause occurs at about 20 years of age (14). At this age, there are also significant hormonal changes such as increased levels of follicle stimulating hormone (FSH) and a fall in oestrogen level. Despite these advantages primates are too dangerous, too expensive, and difficult to maintain to constitute the main model for the study of osteoporosis. The use of primates for scientific research also raises specific objections from the public. Dog as a model after ovariectomy was used in many previous studies of metabolic bone disease (1, 12, 19, 25); however, its usefulness for the study of bone remodelling and the mechanical properties of human bone remains controversial, since ovariectomy in dogs has little effect on the reduction of bone mass (28). In contrast to human and primates, which are polyoestral, dogs (in most cases) are dioestral (7, 28). Although dogs may have limited use as a model for bone loss caused by oestrogen deficiency, they appeared to be very useful for the assessment of general aspects of the human skeleton, bone growth, and arthroplasty (26, 28). Cat may be a suitable model for the study of osteoporosis because of its size and ease of maintenance. Moreover, it is quite easy to induce osteopenia in them by reducing activity and feeding food low in calcium or high in phosphorus. However, due to the fact that cats are regarded as companion animals, their use as experimental animals is a social problem.

Rats and mice are commonly used as experimental animals for the study of osteoporosis (28). The advantages of these models are primarily low price, ease of maintenance, and the fact that the public is accustomed to the role of rodents in experiments. However, due to lack of the Haversian systems in cortical bone, and no osteoblast dysfunction in late stages of oestrogen deficiency, rats are not suitable animals for studying the effects of ovariectomy on bone

mass (18, 28, 30). Rabbits, due to their short period of development and a faster rate of change in bone than primates, have been used to determine the effect of oestrogen on bone density during their development (8, 28). A miniature pig is a species in which the bone metabolism, oestrous cycle, and function of the gastrointestinal tract testify in its favour as an animal model for the study of osteoporosis. However, the high cost, maintenance difficulty, and small size have meant that this model has not been accepted. Sheep is a large animal model that has been recognised by researchers as a model for the study of osteoporosis in humans. It is a gentle animal, easy to maintain, and its size and skeletal structure are generally comparable to that of a human. The histological construction and remodelling processes occurring in sheep bone are similar to those occurring in humans. In contrast to humans, sheep have yearly anoestrus lasting one to two months. During the rest of the year, the production of oestrogen is relatively high (19). In sheep, as in rats (13, 29), the transition into the menopause period in middle age, which is characterised by an acceleration of bone loss, cannot be unambiguously determined.

Due to the fact that osteoporosis does not occur naturally in animals, in sheep it has to be induced. In order to induce osteoporosis, scientists use many methods. The most important are a diet low in protein and minerals, limited movement, limited access to sunlight, ovariectomy, and administration of glucocorticoids. All of these actions are a reflection of what happens to the human body in adulthood: weaker absorption and assimilation of nutrients, reduced activity, avoiding the sun, menopause or andropause, and hormonal therapy.

The purpose of this study is to present a safe and effective way to induce osteoporosis in sheep.

Material and Methods

The experimental groups consisted of female merino sheep, five to six years of age, divided into three groups. The first group (n = 3) consisted of negative controls (KN). They were fed a diet low in nutrients and minerals and had limited access to sunlight and limited activity. Sheep of the second group (n = 3), positive controls (KP) were ovariectomised in addition to the above restrictions. The animals of the third group (n = 15) had the osteoporosis-provoking limitations used in the first group, ovariectomy, and steroids. All animals were weighed and placed in small boxes (10 m^2) at the beginning of the experiment. The diet consisted of hay administered twice a day and water *ad libitum*. The ovariectomy was performed after a two week acclimation period. In addition, biopsies from the left iliac crest were taken from all animals for quantitative computed tomography (QCT) testing (Fig. 1).

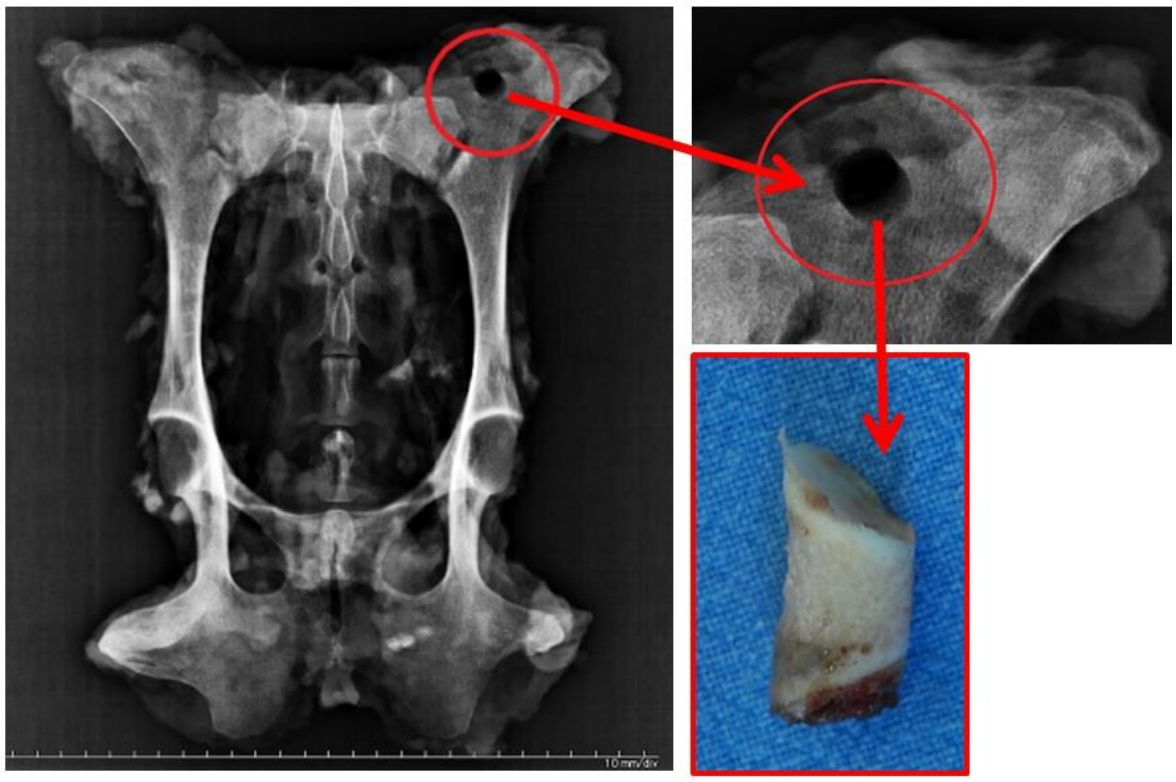


Fig. 1. Radiograph of the pelvis of a sheep with the marked site of bone biopsy (ilium plate - red arrow), and bone sample

The animals were premedicated with a mix of medetomidine (Domitor, 1 mg/mL) + midazolam (Midazolam, 1 mg/mL) at a dose of 10 µg/kg medetomidine + 0.1 mg/kg b.w. midazolam in one syringe, i.m. After inserting a catheter into a vein (lat. vena cephalic antebrachii) general anaesthesia was induced with propofol 20 mg/mL, at a dose of 1 mg/kg b.w., i.v. Then analgesia was achieved by spinal anaesthesia with 2% solution of lignocaine (2% hydrochloricum lignocainum, 20 mg/mL), at a dose of 1 mg/kg b.w. After surgery, the animals were secured using buprenorphine (Vetergesic, 0.3 mg/mL) at a dose of 20 µg/kg b.w., and flunixin (Finadyne, 50 mg/mL) at a dose of 0.5 mg/kg b.w. Ovariectomy was performed in the dorsal position in the midline laparotomy, and biopsies were taken in the lateral position. One month after surgery, the treatment with glucocorticoids started in the third group. Each sheep received four intramuscular doses of 150 mg of methylprednisolone, at intervals of 20 d. In total, each animal received 600 mg of methylprednisolone within 80 d. Two weeks after termination of glucocorticosteroid administration, biopsies from the right iliac crest for QCT tests were collected.

The obtained data was processed with the Shapiro-Wilk test. ANOVA analysis was also used. Statistical analysis was performed using the programme Origin 8.0 (OriginLab, USA).

Results

Results from the QCT study of bone biopsies expressed in Hounsfield units are shown in Table 1. It was found that the decrease in bone density in the KN group was minimal. In the group KP, the declines were slightly higher, but without statistical significance. In the group KS, where in addition to ovariectomy the steroid drugs were applied, the losses were more than three times higher.

Table 1. Results from the QCT study of bone biopsies taken from the iliac crest and expressed in Hounsfield units (HU). Measurement 1 - the beginning of the experiment; measurement 2 - after the administration of steroids

Group and no. of sheep	Measurement 1	Measurement 2
KN 1	1861	1897
KN 2	2223	2127
KN 3	1956	1956
KP 1	1951	1904
KP 2	2080	1885
KP 3	2508	1945
KS 1	2639	2229
KS 2	2234	1922
KS 3	2156	1836
KS 4	1590	1480
KS 5	2729	2055
KS 6	1961	1728
KS 7	1781	1636
KS 8	2047	1813
KS 9	2312	1835
KS 10	1800	1593
KS 11	1934	1726
KS 12	2005	1900
KS 13	2305	2200
KS 14	2334	2103
KS 15	1713	1603

Discussion

Despite many clinical observations and experimental studies, the problem of osteoporosis is still needful of work and of interest to many fields of science. In the study of the changes taking place during the process of bone remodelling after ovariectomy and steroid administration, sheep were used, since these animals provide an excellent large animal model for research on bone growth and remodelling evaluation, as Pastoureau *et al.* (21) and Chavassieux *et al.* (4) observed and as was confirmed in the paper. The literature provides a variety of information on doses and frequency of administration of steroid drugs to accelerate and enhance the onset of osteoporosis. Augat *et al.* (2) and Ding *et al.* (6) in their study used a regimen of daily application of a steroid drug in a dose of 0.6 mg/kg. Lill *et al.* (15) conducted experiments in a dose increased to 15-25 mg per day. Each of these protocols induced a significant and visible impact on the mass, structure, and remodelling of bone. However, the supply of such high doses causes many side effects such as serious systemic infections or loss of fleece (6, 15). After analysis of the literature data dealing with the dose administration regimen, methylprednisolone was chosen, administered in a dose of 150 mg/sheep at long intervals (every 20 d). The drug was administered intramuscularly, not subcutaneously as in other studies (23). This method was chosen due to the fact that steroid drugs given intramuscularly are absorbed faster than after subcutaneous administration. Furthermore, intramuscular administration is indicated when long drug action time is expected. The interval between administrations of each consecutive dose being set at 20 d indicates a long-acting drug. Moreover, subcutaneous administration may lead to skin necrosis at the site of injection, which would have a negative impact on the organism. The literature offers various opinions concerning the specifying of the appropriate experiment duration to achieve the expected reduction in bone mass and changes in bone microstructure in sheep after ovariectomy. A four-month period of research proved to be sufficient to achieve the objectives in the group which underwent ovariectomy and the group treated additionally with steroids. Oheim *et al.* (20) claimed that ovariectomy in sheep leads to a rapid and significant loss of bone mass as early as three months after surgery, but according to Turner *et al.* (27) a reduction in bone mass can be achieved after six months. Some studies suggest that it is after a period of more than 12 months following surgery that ovariectomy may cause a significant loss of bone mass and changes in bone microarchitecture (19). According Chavassieux *et al.* (4) and Ding *et al.* (6), the introduction of corticosteroids in sheep leads to faster and far more significant loss of bone mass, microarchitecture, and disorders in biomechanics. The achieved changes are comparable to the changes described in humans.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: The studies were approved by the Local Bioethics Committee.

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