Comparison of Measuring Methods for Osteopenia Using Two Different Rat Models

Shigeyuki Kanai
Research Institute of Oriental Medicine
Kinki University School of Medicine
Department of Anatomy,
Kansai College of Acupuncture Medicine
Norimasa Taniguchi
Department of Development Research
PIP-FUJIMOTO Corporation

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Introduction

Bone diseases such as osteoporosis and osteoarthritis are regarded as age-associated diseases, and occur in a significantly increasing number of patients, primarily due to longevity. Participation rates in bone mineral density (BMD) mass screenings are increasing by means of several noninvasive methods and analytical systems. However, these methods and systems have been not comparatively evaluated using an animal model for osteopenia. Therefore, we evaluated comparatively the measuring methods for osteopenia by dual-energy X-ray absorptiometry (DEX) and computed X-ray absorptiometry (CXD) respectively, using two different rat models, ovariectomized (OV) rat and adjuvant arthritis (AA) rat.

Materials and methods

Animals

Wistar rats and Sprague-Dawley (SD) rats were obtained from Japan SLC, Inc (Hamamatsu Japan). The animals were given free access to solid rodent chow (CE-2: Nihon Clea, Osaka, Japan) and tap water ad libitum. Animals were housed in a room with a 12h light/dark cycle, at a temperature of 23.0±1.0°C and a relative humidity of 50±5%. Rats were acclimated to the environment for one week before the experiment.

Preparation of animal models for osteopenia

In this experiment, 12 female Wistar rats (6 weeks old, 120-140g) and 12 female SD rats (6 weeks old, 120-140g) were used. Four groups of rats (six rats/group) were categorized to carry out this experiment. Group one Wistar rats were OV and these OV rats were subsequently bred for subsequent 24 weeks to induce systemic osteoporosis. Group two Wistar rats were untreated.
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and these normal rats were bred for 24 weeks as the control for group one. In group three SD rats, AA was induced by intradermal injection (0.5mg/rat) of heat-killed *Mycobacterium butyricum* (Wako Pure Chemical Industries, Ltd., Osaka, Japan) suspended in paraffin oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan), into the base of left hind leg. After injection, these AA rats were bred for 24 weeks to induce extensive bone resorption in the distal diaphysis of the tibia. Group four SD rats were untreated and these normal rats were bred for 24 weeks as control of group two.

**Measurement of bone mineral density**

Measurement of BMD was performed on all 30-weeks-old rats. After being deeply anesthetized with ether, the animals were sacrificed. The left tibia was isolated from each animal and its BMD was measured by DEX as well as CXD, respectively. DEX and CXD were analyzed using DCS-600R Dichroma scan (Aloka Co., Ltd., Tokyo, Japan) and Bonalyzer II (Teijin Limited, Tokyo, Japan), respectively. After measurement of BMDs by both analyzers, correlation between BMDs was evaluated.

**Statistical Analysis**

All values were expressed as means ± SE. Difference analysis on each value between each group was performed using Wilcoxon signed rank test for the paired comparisons. The results of linear regression analysis were demonstrated as Pearson correlation coefficients. Probability levels less than 0.05 were considered to significant differences.

**Results**

1) a) Mean BMDs of tibiae in OV rats were decreased about 11% compared to those of their respective controls by DEX and about 30% by CXD.

b) Mean BMDs of tibiae in AA rats were decreased about 10% compared to those of their respective controls by DEX and about 13% by CXD (Fig. 1).

2) BMDs of tibiae in all rats showed significant positive correlation between DEX and CXD (Fig. 2).

**Discussion**

Bone formation and resorption are ongoing phenomena. When bone resorption equals bone formation, bone mass remains stable. When resorption exceeds formation, bone mass is reduced a process that leads to osteopenia or osteoporosis. Osteopenia is reduced bone mass and osteoporosis is reduced bone mass with resultant fractures. Reduced bone mass may be postmenopausal or related to ovarian failure (type I osteoporosis), it may be age-related (type II osteoporosis), or it may result from several other etiologic factors (secondary osteoporosis). As an experimental model of postmenopausal osteoporosis or osteopenia, OV is frequently performed to induce estrogen deficiency-related bone loss. However, there is considerable evidence that rheumatoid arthritis (RA) induces osteoporosis and osteopenia in humans. Osteopenia via RA in humans might be associated with several complicated factors, e.g., reduced physical exercise due to pain, steroid administration to treat RA, malnutrition, and dyshormonism. There is also
Fig. 1 BMDs for osteopenia by DEX and CXD in OV rats and AA rats. All values were expressed as means±SD. *p<0.05; **p<0.01, significantly different between each group.

Fig. 2 Correlation between BMDs for osteopenia by DEX and CXD in OV rats and AA rats.

Evidence that strengthening exercises may lead to an increase in BMD in the bones to which the exercised muscles are attached. In addition, the nonphysiological mechanical stimulation, in the form of low intensity vibration (frequency: 50 Hz, acceleration: 2g, 30min/day for 5 days/week) significantly prevented early bone loss after OV11.

We observed that long-term immobility via severe osteoarthritis induced osteopenia in chronic AA rats up to 6 months post-OV as an experimental model of RA. In these chronic AA rats, significant reduction of BMD was detected by DEX as well as CXD (data not shown). Moreover, extensive bone resorption is observed in the distal diaphysis of the tibia of AA rats, accompanied with clusters of numerous multinucleated giant cells (MGCs) as well as bone-resorbing osteoclasts4.

Recently, quantitative X-ray computed tomography (QCT), which can assess the exact bone
density in a more restricted area, is recommended for accuracy and precision in measurement of BMD\[1\]. Thus far, however, DEX and CXD are more prevalent relative to QCT, mainly due to availability, usability and low exposure dose. Our results demonstrate that both analytical methods of safe and easily applicable measurement may provide sufficiently reliable and suitable tools assess BMD for osteopenia.

Further study will focus on testing other regions of the body, e.g., femur and calcaneus, and other methods, e.g., ultrasound to determine its usefulness in humans.

Conclusions

AA rats might be one of the useful experimental models for osteopenia in accordance with OV rat. Furthermore, both analytical methods, DEX and CXD were a reliable and suitable tools for assessing of BMD for osteopenia.

References

実験的骨減少症ラットに対する DEX 法及び CXD 法の検討

新潟大学東洋医学研究所
関西薬科短期大学解剖学
金 井 宏 行
ピップフジモト株式会社開発研究部
谷 口 典 正

実験的骨減少症モデルと考えられている卵巣摘出（OV）ラットと実験的関節症モデルであるアジュバント関節炎（AA）ラットの脛骨の骨塩量を DEX 法と CXD 法にて測定した。OV ラット、AA ラットの骨塩量は、DEX 法のみならず CXD 法においても有意にコントロール群に比べて減少を示した。また、DEX 法と CXD 法による骨塩量には、有意に相関が認められた。これらの結果から AA ラットも実験的骨減少症モデルに充分なりうると考えられ、CXD 法も骨減少の評価に有用であることが示唆された。