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A systematic review of current osteoporotic metaphyseal fracture animal models

R. M. Y. Wong,*
M. H. V. Choy,*
M. C. M. Li,
K-S. Leung,
S. K-H. Chow,
W-H. Cheung,
J. C. Y. Cheng

The Chinese University of Hong Kong, Shatin, Hong Kong

- R. M. Y. Wong, BCMPH, MBChB, MRCS(Ed), Resident Doctor, Department of Orthopaedics and Traumatology, Prince of Wales Hospital Authority, 30-32 Ngan Shing Street, Shatin, Hong Kong and Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Shatin, Hong Kong
- M. H. V. Choy, BSc, PhD Candidate,
- M. C. M. Li, BEng, MSc, Research Assistant,
- K-S. Leung, MBBS, MD, FRCS(Edin), FHKCOS, FHKAM(Orth Surg), FACS, Dip. Biomechanics, Emeritus Professor of Orthopaedics and Traumatology,
- S. K-H. Chow, BSc, MSc, PhD, Research Assistant Professor,
- W-H. Cheung, BSc(Hons), PhD, PgCert(Biostatistics), Associate Professor,
- J. C. Y. Cheng, MD, MBBS, FRCS(Edin), FRCS Ortho(Edin), FHKCS, FHKCOS, FHKAM(Ortho Surg), Professor and Chairman of Orthopaedics and Traumatology, Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Shatin, Hong Kong.

Correspondence should be sent to W-H. Cheung;
email: Louis@ort.cuhk.edu.hk

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Objectives

The treatment of osteoporotic fractures is a major challenge, and the enhancement of healing is critical as a major goal in modern fracture management. Most osteoporotic fractures occur at the metaphyseal bone region but few models exist and the healing is still poorly understood. A systematic review was conducted to identify and analyse the appropriateness of current osteoporotic metaphyseal fracture animal models.

Materials and Methods

A literature search was performed on the Pubmed, Embase, and Web of Science databases, and relevant articles were selected. A total of 19 studies were included. Information on the animal, induction of osteoporosis, fracture technique, site and fixation, healing results, and utility of the model were extracted.

Results

Fracture techniques included drill hole defects (3 of 19), bone defects (3 of 19), partial osteotomy (1 of 19), and complete osteotomies (12 of 19). Drill hole models and incomplete osteotomy models are easy to perform and allow the study of therapeutic agents but do not represent the usual clinical setting. Additionally, biomaterials can be filled into drill hole defects for analysis. Complete osteotomy models are most commonly used and are best suited for the investigation of therapeutic drugs or noninvasive interventions. The metaphyseal defect models allow the study of biomaterials, which are associated with complex and comminuted osteoporotic fractures.

Conclusion

For a clinically relevant model, we propose that an animal model should satisfy the following criteria to study osteoporotic fracture healing: 1) induction of osteoporosis, 2) complete osteotomy or defect at the metaphysis unilaterally, and 3) internal fixation.

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Keywords: Metaphyseal Fracture, Animal Model, Osteoporosis, Systematic Review

Article Focus

- Most osteoporotic fractures occur at the metaphyseal bone region but few models exist and the healing is poorly understood
- Systematic review to identify and analyse the appropriateness of current osteoporotic metaphyseal fracture animal models

Key Messages

- Complete osteotomy models are most commonly used and are best suited for the investigation of therapeutic drugs or noninvasive interventions
- The metaphyseal defect models are best suited for the study of biomaterials, which are associated with complex and comminuted osteoporotic fractures

Strengths and limitations

- Updated review of currently available models
- Due to the heterogeneity of the studies, pooled analysis was not feasible

Introduction

Osteoporosis is a major medical and socioeconomic threat characterized by a systemic impairment of bone mass, strength, and microarchitecture. The skeletal disorder predisposes patients to increased risk of fragility fractures. There are approximately 2.5 million osteoporotic fractures each year in the United States, with costs estimated at $15 billion USD in 2010 and projected to reach $25 billion USD by 2025.[1] In 2000, an estimated 9.0 million osteoporotic fractures occurred worldwide, with the numbers continuously rising.[2] The lifetime fracture risk of osteoporotic patients reaches as high as 40%,[3] which is an important cause of morbidity and mortality in an ageing population.

The treatment of osteoporotic fractures is a major challenge, as bone healing is delayed due to the impaired healing properties with respect to callus formation, angiogenesis, and mineralization.[4,5] Failure to unite results in pain, weakness, reduced mobility, and fixation failure; these complications are most common in elderly patients, which can lead to serious detrimental effects to overall health status. Enhancement of osteoporotic fracture healing is therefore critical as a major goal in modern fracture management. The development of an effective animal model for research is essential in this process.

Most osteoporotic fractures occur at the trabecular or the metaphyseal bone region,[6] including the distal radius, proximal humerus, proximal femur, and vertebral bodies.[7,8] Despite the evidence, most preclinical studies have concentrated on the healing of osteoporotic diaphyseal femur or tibia fractures with intramedullary fixation, often based on the model put forward by Bonnarens and Einhorn[9] in 1984. However, it is well known that metaphyseal and diaphyseal fractures heal by completely different mechanisms.[10] This animal model has therefore faced criticisms related to its clinical relevance, leading to the recent development of more appropriate models.

There are very few of these newer models, and the healing of metaphyseal fractures is still poorly understood. The purpose of this systematic review was to identify and characterize the appropriateness of the available metaphyseal fracture animal models reported for osteoporosis research.

Materials and Methods

Search strategy. The Pubmed, Embase, and Web of Science databases (date last accessed 07 May 2017) were searched. The keywords used for the search criteria were “metaphys*” AND “animal model” AND “fracture” AND “osteoporo*”.

Search criteria. The inclusion criteria were: 1) preclinical studies, 2) use of animal model, 3) fractures performed at the metaphysis, and 4) study on fracture healing.

The exclusion criteria were: 1) review paper, 2) lack of osteoporosis induction, 3) no radiological imaging or histological analysis for fracture healing, 4) lack of control group, and 5) conference/abstract publication.

Selection of studies. Two independent reviewers performed the selection process on three databases. Each reviewer screened the titles and abstracts of each published study. Articles were selected based on the inclusion and exclusion criteria. Each article was reviewed and any disagreement was resolved by consensus and discussion.

Data extraction. For eligible studies, the two reviewers extracted information on: 1) animal used; 2) osteoporosis induction and method; 3) site and type of fracture; 4) type of fixation; 5) fracture healing results; and 6) up-to-date literature on the utility of the animal model.

Data analysis. Due to the large variation in animal models and methodology, a qualitative review was performed.

Results

A total of 41, 84, and 53 studies were identified from PubMed, Embase, and Web of Science, respectively. All duplicate entries were removed, leaving 127 records. Each title and abstract was reviewed and 97 records were excluded. Upon detailed review of each study in full text, an additional 11 were excluded. One of these studies did not have induction of osteoporosis.[11] Three studies performed fractures in the diaphysis.[12-14] Three lacked a control group in the study design.[15-17] Two studies were not related to fracture healing.[18,19] Two studies did not have radiological imaging or histological analysis of fracture healing.[20,21] Our results show a total of 19 studies for our systematic review (Fig. 1).

Characteristics of the papers. The 19 studies were published from 2010 to 2016 (Supplementary table i). All studies were preclinical studies with metaphyseal fracture models and induction of osteoporosis, performed in the rat,[7,10,22-36] sheep,[37] and goat.[38]

Induction and methodology of osteoporosis. All 17 rat studies performed bilateral ovariectomy to induce osteoporosis. One study performed ovariectomy on Chinese mountain goats and another study performed hypothalamic-pituitary disconnection (HPD) on adult Merino sheep. Supplementary table i summarizes the osteoporotic induction methods and confirmation of osteoporosis.

Out of the 19 studies (Supplementary table i), nine performed analysis by micro-CT, pQCT (peripheral quantitative computed tomography), or DXA (dual-energy X-ray absorptiometry) to confirm osteoporotic induction. Seven studies were based on previous literature that confirmed osteoporosis. Three had created a new type of osteoporotic model, to simulate the early phase of osteoporosis.

Location and type of fracture. All fractures from the 19 studies were performed at the metaphyseal region of bones. Three were drill hole defect models, one was a partial osteotomy model, three performed

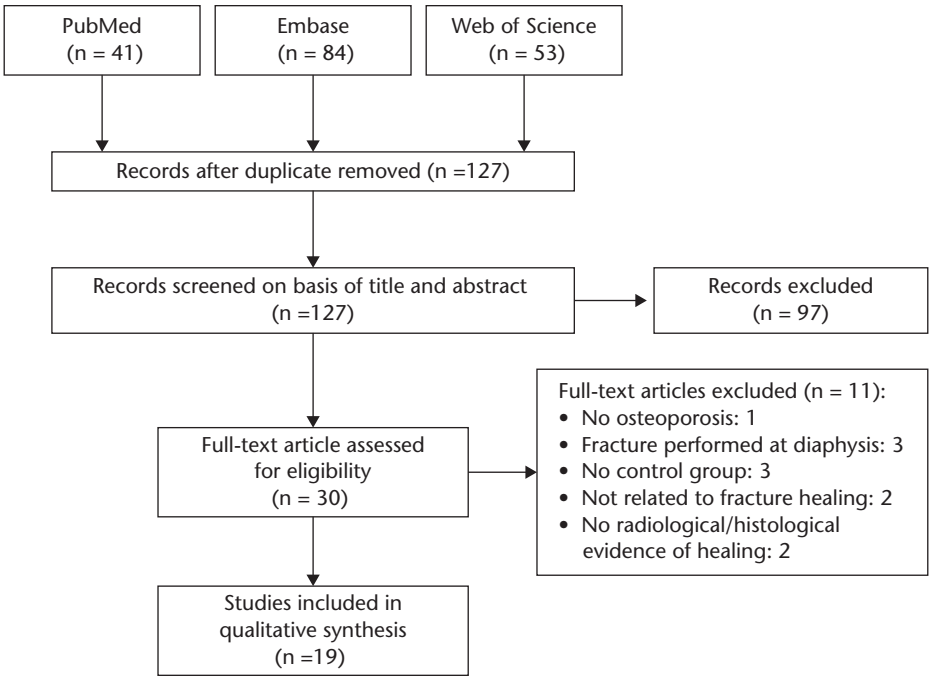

Fig. 1

Flowchart of study selection

fracture defect models, and 12 were complete osteotomy fracture models. The details are summarized in Supplementary table i.

Radiological and histological evidence of healing. All studies reported adequate fracture healing. Supplementary table i summarizes the radiological and histological findings for all 19 studies.

The interventions assessed with current osteoporotic animal models. The most commonly used osteoporotic metaphyseal fracture model was the bilateral osteotomy on the proximal tibia of ovariectomized rats, originated by Stuermer et al,[10] which was used by 11 published studies.[10,25-31,33,34,36] All 11 studies that used the bilateral osteotomy model investigated potential therapeutic drugs or noninvasive interventions. The unilateral complete osteotomy was used to investigate potential therapeutic agents to promote osteoporotic fracture healing. The three defect models enabled the study of biomaterials, while the three drill hole defect models were used to study biomaterials or therapeutic drugs. The partial osteotomy model has not yet been used to study interventions. Supplementary table i summarizes the details on the utility of each model.

Discussion

Previous models have concentrated on diaphyseal fractures, despite evidence that they heal by completely different mechanisms to metaphyseal fractures.[39,40] Metaphyseal fractures heal in a rapid fashion. Chen et al[41] have shown that there are several distinct histological stages in metaphyseal fracture healing with "cellular activation and differentiation, formation of woven bone, transformation of woven bone into lamellar bone and further remodeling". On the other hand, diaphyseal fractures heal with a complex multistep process, in which both intramembranous and endochondral ossification occur to complete the process.[39,41] Animal models using diaphyseal fractures are therefore considered not adequate for osteoporotic fracture research.[10]

Different osteoporotic induction methods were used in 19 studies in this systematic review. Most authors in this review have used the widely accepted ovariectomized rat model to produce this effect. The ovariectomized rat is the Food and Drug Administration (FDA) approved animal model to study osteoporosis.[42] Rats are of low cost, require little maintenance, are easy and safe to handle, and have high reproducibility. It is known that rats reach sexual maturity at 2.5 months of age, and that their skeleton is considered mature after the age of 10 months.[43] Both skeletally mature and immature rats can be used for the induction of osteoporosis. The use of the skeletally immature rat is appropriate for osteoporotic research as a low peak bone mass is achieved, which is a high-risk factor for human osteoporotic fractures.[44] After ovariectomy in skeletally immature rats, the circulation of oestrogen is reduced and primary osteoporosis Type 1 and postmenopausal status are induced.[45] In skeletally mature or aged rats, the process causes

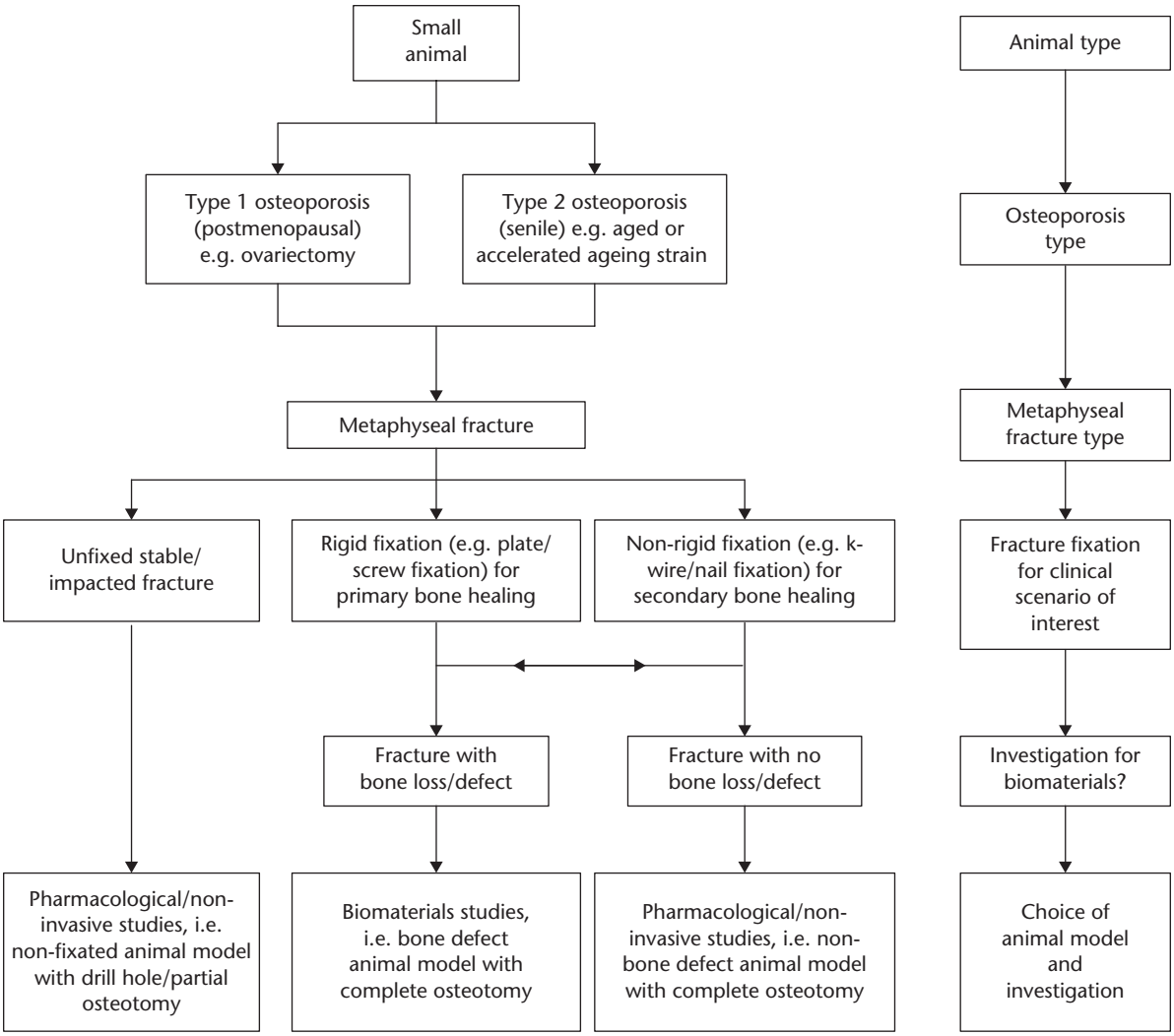

Fig. 2

Algorithm for small animal models for the investigation of osteoporotic fracture healing. Fracture types include complete osteotomy, bone defect, drill hole, and partial osteotomy.

cancellous and endocortical bone loss, which exhibits primary osteoporosis Type 2 or senile osteoporosis.[44,45] It is also well-established that osteoporosis occurs within two to three months postovariectomy, and studies have also shown that diet modifications can complement this process.[10,46] Therefore, osteoporotic induction is adequate for the current rat models.

Osteoporotic models using larger animals, including goat and sheep, have also been described for osteoporotic research, but are considered to be second-line choices by the FDA. These models are less efficacious due to cost and availability, housing and spatial requirements, manageability, and reproducible results.[47] Therefore, sample sizes are much lower compared with those in rodent models. However, these animals have the advantage of having haversian systems in bones that resemble those of humans.[47] The current FDA-preferred osteoporotic induction method is by ovariectomy. In fact, the hypothalamic-pituitary disconnections performed by Bindl et al[37] have unwanted side effects, including polydipsia and polyuria from diabetes insipidus.

Current established and well-accepted parameters for osteoporosis assessment include the use of bone densitometry, such as dual-energy X-ray absorptiometry (DXA) scan, pQCT, and micro-CT, to evaluate the bone mineral density.[43] Currently, DXA is the most widely validated technique to measure BMD (bone mineral density), which is the benchmark parameter for reference as defined by the World Health Organization (WHO). Other structural CT parameters can further assess and support architectural changes.

It is well known that the biomechanics and bone tissue quality of osteoporotic bone is significantly different to those of normal healthy bone. Most importantly, if osteoporotic fracture healing is the target of interest, the lack of induction of osteoporosis renders the model clinically irrelevant. Therefore, the induction of osteoporosis is essential in osteoporotic fracture studies.[7,10,48]

A few studies were simplified metaphyseal fracture models, including drill hole defects[23,35,38] and partial osteotomy.[37] Understandably, these drill hole defect and partial osteotomy models do not require fixation, and do not represent a clinically relevant scenario. The healing process is also very different from a complete fracture.[38] Animal models that characterize a clinical fracture would need to create a complete discontinuity of the bone.[48] On the contrary, simplified models with drill holes and partial osteotomies are easy to perform and have high percentages of success.[23,37,38] Furthermore, drill hole defects have allowed the investigation of therapeutic drugs and biomaterials.[23,38]

All three bone defect models had complete discontinuity. These provide a clinically relevant model with the addition of osteoporosis and adequate stability with plate fixation similar to the clinical situation.[48] Large metaphyseal defects are often accompanied with bone graft or substitutes during surgery, and the healing is evidently different to that of normal metaphyseal fractures.[7] These models are created to best serve the study of biomaterials in the enhancement of osteoporotic fracture healing as stated by Alt et al.[7] However, the use of implants with plates and screws increases the cost of the study and has potential complications, such as more technically difficult fixation.[32]

All 12 complete osteotomy models in this review had appropriate osteoporotic induction and complete discontinuity of bone during the osteotomy. However, the 11 studies by Stuermer et al,[10,27,28,33] Kolios et al,[25,26,29] and Komrakova et al[30,31,34,36] performed bilateral proximal osteotomies on rats. Complete osteotomies are clinically relevant, but it is rare that both limbs are affected in clinical cases. Bilateral osteotomies may need to be avoided for ethical reasons if there is significant negative influence on the weight-bearing status of the animal during the healing phase, which would subsequently affect results.[49] It would therefore be more appropriate for animal models to have a unilateral fixation instead. Ibrahim et al[2] was the only study with a complete osteotomy unilaterally with fixation, but the authors did not comment on the success rate. Based on current literature, complete osteotomy models are appropriate for the investigation of potential therapeutic osteoporotic drugs and noninvasive interventions.

Although there are several metaphyseal models for the analysis of osteoporotic healing, there is room for improvement. Following analysis of the current models, we have derived a recommendation for future models. For a clinically relevant model, we propose that an animal model should satisfy the following criteria to study osteoporotic fracture healing: 1) induction of osteoporosis, 2) complete osteotomy or defect at the metaphysis unilaterally, and 3) internal fixation. Furthermore, in order to match clinical scenarios, we have created an algorithm for investigators to use in deciding the animal model of their interest (Fig. 2).

Our past research has focused on osteoporotic fracture healing with a diaphyseal animal model.[4,5,50] Our previous results show that osteoporotic healing was significantly delayed in terms of active callus formation, mineralization, angiogenesis and remodelling. However, a change in the animal model to a metaphyseal fracture following our new proposed criteria would provide a more accurate depiction of osteoporotic fracture healing. This is essential for quality studies, and for the establishment of future clinical interventions.

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Author Contribution

- R. M. Y. Wong: Principal investigator, Designing and carrying out the study, Writing the manuscript.
- M. H. V. Choy: Co-first author, Reviewing the manuscript, Assisting in carrying out the study.
- M. C. M. Li: Reviewing the manuscript, Assisting in carrying out the study.
- K-S. Leung: Investigator, Designing and supervising the study, Reviewing the manuscript.
- S. K-H. Chow: Investigator, Designing the study, Reviewing the manuscript.
- W-H. Cheung: Investigator, Designing and supervising the study, Reviewing the manuscript.
- J. C. Y. Cheng: Investigator, Designing the study, Overall supervision of the study, Reviewing the manuscript.

- *R. M. Y. Wong and M. H. V. Choy contributed to this article equally.

Conflicts of Interest Statement

- The authors declare no conflict of interests.